Abstract

Quantitative Semiconductor Nanosensors

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Nanoscale Field Effect Transistors (FETs) have emerged as a promising technology for ultrasensitive and unlabeled diagnostic applications; however, their practical use has been hindered by the lack of methods for quantitative sensing and more importantly the detailed understanding of the relationship between sensitivity and dimensional scaling. In this work, we explore the fundamental relationship between the electrical and sensing properties of nanoscale FET and introduce methods for quantitative sensing and internal sensor calibration. By developing top-down fabrication processes for Complementary Metal Oxide Semiconductor (CMOS) compatible Silicon-On-Insulator (SOI) nanowire and nanoribbon FET arrays, we demonstrate multiplexed detection of bimolecular species which ultimately leads to an internal calibration scheme for molecular quantification. We apply this method to two different detection schemes. First, we investigate label-free detection of biomolecules – e.g., cancer biomarkers and successfully demonstrate calibration curves and quantification with lower relative standard error of the mean compared to traditional Enzyme Linked ImmunoSorbent Assay (ELISA). Second, we investigate the relationship between nanoscale FET sensor operation and size scaling by comparing experimental data and developing new theoretical models. Finally, we introduce the Debye Length modulation technique which allows probing of the surface functionalization and determining the average spatial extent of bound surface charge.
For the first time since the foundation of the FET based sensor field we demonstrate their application as quantitative tools. This step is our determination to bring the field forward towards creation of a powerful point-of-care diagnostic tool.
Quantitative Semiconductor Nanosensors

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To Mom and Dad
Gutta cavat lapidem non vi sed saepe cadendo.

Publius Ovidius Naso
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In the end, to all future graduate students (and readers) who, by sheer accident or true inquisitiveness might read a few paragraphs from this manuscript, I say (taken from the movie “Sucker Punch”):

> “Who honors those we love with the very life we live? Who sends monsters to kill us... ...and at the same time sings that we'll never die? Who teaches us what's real and how to laugh at lies? Who decides why we live and what we'll die to defend? Who chains us? And who holds the key that can set us free? It's you. You have all the weapons you need. Now fight!!!”

Aleksandar Vacic
New Haven, Connecticut
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1. Introduction

The ability to sense biomolecules using direct electronic label-free detection in disparate environments is of great interest for biomedical, clinical, pharmaceutical and defense research. Recent advancements in nanofabrication techniques offer a promise for delivering portable electronic platforms capable of rapid, ultrasensitive, low-cost, low-powered and multiplexed identification of various biomolecular species. Compared to the current cutting-edge techniques such as surface plasmon resonance, radio-tags and DNA microarrays, electronic detection allows integration of sensor arrays with data processing components (registers, amplifiers, analog-to-digital converters…), on-chip multiplexing and microfluidic integration. Over the past decade semiconducting nanowires, configured as field effect transistors (FETs), have demonstrated great promise to satisfy these demands and reach sensitivities that are compared to or better than these optical techniques.

The idea of using FETs for detection of biomolecules was first demonstrated three decades ago. An Ion-Sensitive FET (ISFET) (Bergveld and Sibbald, 1988) is similar to a conventional Metal-Oxide-Semiconductor FET (MOSFET), however, instead of a metal gate that is used to turn the device on and off, an ISFET lacks the metal gate, thus allowing the oxide layer to be exposed to the electrolyte solution. This electrolyte-oxide interface contains dangling bonds i.e. binding sites which act as a gate whose electrostatic potential can be modulated by the binding of charged species. Binding of ions (charged molecules, proteins, etc.) from the solution to the ISFET surface causes changes in the semiconductor surface potential and, therefore, modulates device current. A typical application of an ISFET for biomolecular detection is achieved by surface modification using enzymes – ENFETs (Schoot and Bergveld, 1988), where bound enzyme
catalyzes chemical reaction which causes a local pH change. The major drawback of this technology is its reliance on enzymatic activity which greatly depends on environment conditions (temperature, buffer ionic strength, etc.) and therefore device lifetime and robustness.

Nanowire FETs, first introduced in 2001 (Cui et al., 2001), demonstrated the much desired ability for detecting molecules at clinically relevant levels (Zheng et al., 2005). As demonstrated by semi-empirical approaches (Liu et al., 2003), a generally accepted explanation for the increased sensitivity of nanoscale sensors is due to increased surface-to-volume ratio (Fan and Liu, 2006). Nanoscale FETs based on chemical-vapor deposition (Wagner and Ellis, 1965) CVD- grown nanowires, have been used for detection of small molecules, proteins, biomarkers and viruses, both for complementary and multiplexed sensing. However, significant variation in electrical characteristics (Stern et al., 2005) and difficulties to incorporate CVD grown nanowires in existing top-down fabrication techniques (McAlpine et al., 2007) still represent major shortcomings of this technology.

Improvements in quality of silicon-on-insulator wafers (Bruel, 1995) and processing techniques (Colinge, 1991) gave a significant boost to the use of top-down fabrication techniques. In these processing schemes a device nano-channel is formed by either electron-beam (EBL) or by deep ultraviolet (DUV) lithography, followed by dry, reactive ion etching (RIE) (Pui et al., 2009). Recent experimental studies (Rajan et al., 2010) on 1/f noise of the RIE defined devices have demonstrated a significant degradation of nanowire electrical and transport characteristics such as carrier mobility and subthreshold swing as well as an increased number of traps.
On the other hand anistropic wet etching of silicon have demonstrated devices with better electrical characteristics (e.g. subthreshold swing, low-frequency noise, etc.) by preserving the sidewall smoothness and lowering the interface trap density (Rajan et al., 2010).

As mentioned before, a very powerful application of nanosensors is direct detection of biomolecules without the need for pre-labeling (i.e. attachment of fluorophores or radiotags), (Patolsky and Lieber, 2005). Detection of specific molecules via direct electronic method requires the sensors to be functionalized with receptor molecules prior to sensing (Patolsky and Lieber, 2006). The type of target molecules determines the receptor molecules and therefore the surface functionalization scheme.

A major drawback of the bioFET is the lack of calibration methods and the inability to quantify biomolecular species. This problem has prevented bioFET technology to become competitive with the current cutting-edge techniques, such as surface plasmon resonance (Jonsson et al., 1991) or ELISA (Enzyme Linked Immunosorbent Assay) (Engvall and Perlmann, 1972). The need for individual device calibration and the lack of understanding the relationship between sensor response and device electrical characteristics has left the bioFET technology behind its competitors.

In this work we demonstrate a method for device calibration and successfully demonstrate quantification of biomolecules using bioFET technology. Furthermore, using basic ISFET theory we relate device sensing and electrical characteristics, which leads to investigation of sensor response dependence on device size.

We further expand the understanding of Debye screening on label-free detection by exploring this effect on an antigen-antibody system. We term this technique Debye Length modulation
(DLM). Using the proposed method and appropriate theoretical models we extract relevant spatial information about the bound charge and we develop a method that discriminates between the different surface modification schemes.

The methods and results demonstrated in this thesis are directed towards better understanding of bioFET physics and developing of reliable, reproducible and quantitative point-of-care tool that can be used in clinical research.
2. Theoretical Considerations

2.1 The concept of ISFET

In the late 70s a concept of Ion Sensitive FET (ISFET) was introduced. (Stanley D. Moss, 1975). Unlike traditional MOSFET operation which involves application of the external voltage on a gate electrode to control the current of a device, Bergveld (Bergveld, 1981) proposed a device structure in which the metal layer of the gate is substituted with an electrolyte solution which acts as a gate. The electrical circuit in such configuration is completed by adding a solution gate (reference electrode) which would regulate the potential of the electrolyte.

Figure 2.1 Comparative schematics of a Metal-Oxide-Semiconductor Field Effect Transistor (MOSFET) and its bioelectronic counterparty – the Ion Sensitive FET (ISFET).

Figure 2.1 a) shows a typical MOSFET configuration consisting of p-type silicon substrate and highly doped source and drain regions from which carriers are injected. The gate electrode, separated by a thin oxide layer, serves to induce carrier accumulation, depletion or inversion which in turn regulates the carrier current between the source and the drain.
Figure 2.1 b) illustrates a configuration of an ISFET, where the gate electrode is replaced by an electrolyte solution. To regulate the potential of the solution, a reference electrode or solution gate (Pt or Ag/AgCl, (Suzuki et al., 1998)) is added to the system. Any change of the surface potential at the electrolyte-insulator surface will cause accumulation or depletion of carriers in the FET channel and will modulate the device current (Bergveld, 1985). For real applications the surface of the ISFET is usually chemically modified to create active surface (Besselink et al., 2003). One typical modification is done by using 3-aminopropyltrietoxysilane which confers amine functionality to the sensor surface, Figure 2.2 (Wang and Jin, 2004).

![APTES functionalization of Silicon](image)

**Figure 2.2 APTES functionalization of Silicon.**

Binding of molecules to the ISFET surface will change device surface potential therefore causing an increase or decrease of device current (Bergveld and Sibbald, 1988).

Figure 2.3 illustrates the effects occurring at electrolyte-semiconductor interface. At the surface, specific adsorption of charged moieties (ions, proteins,...) occurs by partially freeing the ions of their solvation shell. The smallest distance of molecules from the semiconductor surface is known as Inner Helmholtz plane (IHP) (Israelachvili, 1991). However, due to the charge present at the analyte surface, ionized ionic species from buffer solution form a hydration shell and effectively lower the charge of the molecules. This hydration shell increases the effective radius of the analyte molecules and do not allow them to approach the interface. This smallest average distance is referred to as the outer Helmholtz plane (OHP) (Israelachvili, 1991). The effective
electric field due to the charge screening causes a non-uniform ion distribution and therefore a potential drop close to the interface. The zone between the IHP and OHP is termed the Stern layer, Figure 2.3.

Figure 2.3 The structure of semiconductor-electrolyte interface introducing three main regions in the electrolyte: the inner Helmholtz plane with adsorbed molecules, Stern layer and diffusive or Gouy-Chapman layer.

As mentioned previously, an ISFET does not possess a metal gate and the oxide layer is directly exposed to an electrolyte solution which acts as an all-around gate. To understand the physics behind an ISFET it is important to understand the spatial potential diagram of an ISFET-electrolyte stack which is given in Figure 2.4. Four distinct regions can be recognized:

1) the potential drop at the reference electrode-electrolyte interface $(E_{\text{ref}} + \chi_{\text{sol}} - \chi_M)$

2) the Gouy-Chapman-Stern double layer at the electrolyte-oxide interface $(\psi_0)$,

3) the potential drop in the oxide $(\psi_{\text{ox}})$,

4) the potential drop due to the depletion charges in the semiconductor $(\psi_s)$. 

Figure 2.3 The structure of semiconductor-electrolyte interface introducing three main regions in the electrolyte: the inner Helmholtz plane with adsorbed molecules, Stern layer and diffusive or Gouy-Chapman layer.
Using the energy diagram in Figure 2.4 one can write down an equation for ISFET current similarly to the one for a MOSFET. For the linear region the following equations holds (Bergveld, 1981):

\[ i_{ds} = \mu C_{ox} \frac{w}{L} \left\{ V_{GS} - \left( E_{ref} - \psi_0 + \chi^{sol} - \frac{\Phi_{Si}}{q} - \frac{Q_{ox} + Q_{SS}}{C_{ox}} - \frac{Q_B}{C_{ox}} + 2\phi_f \right) \right\} V_{DS} \left( \frac{1}{2} V_{DS}^2 \right) \]

where \( \mu \) is the carrier mobility, \( C_{ox} \) the capacitance of the oxide layer, \( w \) and \( L \) are width and length of the ISFET, respectively; \( V_{GS} \) is the potential of the reference electrode, \( E_{ref} \) is the reference electrode-electrolyte interface work function, \( \psi_0 \) is the electrostatic potential at the sensor surface, \( \chi^{sol} \) is the surface dipole potential of the solution, \( \Phi_{Si} \) is the silicon work function, \( Q_{ox} \), \( Q_{SS} \) and \( Q_B \) are the charges in the oxide, interface (surface states) and semiconductor, respectively; \( \phi_f \) is the potential difference between the Fermi levels of doped and undoped silicon.
The most important role is played by the surface potential $\psi_0$ which reflects the changes that occur at the sensor surface. Therefore, any binding of charge at the nanosensor surface will cause change of the nanosensor surface potential and will directly affect the current.

For traditional MOSFET device this equation can be written in more compact form (Sze, 1981):

$$i_{ds} = \mu C_{ox} \frac{w}{L} (V_{GS} - V_T) \cdot V_{DS}$$

for the linear regime under the assumption of low source-drain bias i.e. $V_{DS} \ll V_{GS} - V_T$. In case of bioFET, an adjustment can be made by adding the surface potential $\psi_0$ in the equation:

$$i_{ds} = \mu C_{ox} \frac{w}{L} (V_{GS} - V_T + \psi_0) V_{DS}$$

The former equation is fundamental for understanding the basic operation of an ISFET and bioFET.

### 2.2 Site-dissociation theory and pH sensitivity

Generally accepted theory (van Hal et al., 1995) behind the sensitivity of semiconductor-insulator surfaces versus pH changes and charges in electrolyte solutions is due to the existence of surface states at the oxide terminated silicon surface (Niu et al., 1996). The existence of Si-O bonds allows for absorption of a proton from the electrolyte solution and thus formation of neutral silanol groups (van Hal et al., 1995), which can be further protonated to reach a positively charged state. Depending on the pH values of the electrolyte the result can be positive surface charge (low pH values), neutral, or negative surface charge (high pH) (van Hal et al., 1995). Figure 2.5 depicts the basic principle of the site-dissociation theory (Bousse et al., 1983) that is used for description of ISFET operation – silanol groups at the terminating surface of
silicon oxide can be either neutral in the form of hydroxyl groups, positive (proton acceptor) or negative (proton donor). The total amount of charge (positive and negative) will determine the dependence of surface potential $\psi$ of a bioFET on pH value in the bulk of the electrolyte, and the value of $d\psi/dpH$ will determine the sensitivity of a bioFET.

Figure 2.5 The schematics of the semiconductor-electrolyte interface and the site-dissociation model.

To calculate bioFET sensitivity one needs to estimate the average amount of surface charge at the bioFET-electrolyte interface for given conditions. The binding can be quantitatively described using thermodynamic equilibrium equations. The acidic and basic character of the silanol groups can be described by the equilibrium constants (Bousse and Meindl, 1987) $K_a$ and $K_b$. Based on the assumption that sensor surface has only silanol groups that can be protonated and deprotonated the following equations will hold:

\[
Si - OH \leftrightarrow Si - O^- + H^+ \quad \text{and} \quad Si - OH + H^+ \leftrightarrow Si - OH_2^+
\]

with:

\[
K_a = \frac{[Si-O^-][H^+]}{[Si-OH]} \quad \text{and} \quad K_b = \frac{[Si-OH_2^+]}{[Si-OH][H^+]},
\]
where $[Si - OH], [Si - O^-]$ and $[Si - OH_2^+]$ are the per-area concentration of the sites. The concentration of protons $[H^+]$ is the concentration at the sensor surface which is different from the one in the bulk of the solution, $[H^+]_b$ due to the surface potential $\psi_0$. These two variables are related via the Boltzmann equation:

$$[H^+] = [H^+]_b \exp(-\frac{q\psi_0}{k_B T}).$$

The total surface charge at the sensor surface due to protonation and deprotonation of silanol groups is given by:

$$\sigma_0 = e([Si - OH_2^+] - [Si - O^-]) = eN_s(\Theta^+ - \Theta^-),$$

where $e$ is an elementary charge, $N_s$ is the total number of available binding sites per unit area, and $\Theta^+$ and $\Theta^-$ are the fraction of the surface states that bear positive and negative charge, respectively. Calculating the fractions from the equilibrium concentration yields

$$\sigma_0 = eN_s \frac{[H^+]^2 - K_a K_b}{K_a K_b + K_b [H^+] + [H^+]^2} = -e[B]$$

where $[B]$ is the difference between surface concentrations of negative and positive charge. The sensitivity of surface charge to changes of electrolyte pH is defined by

$$\frac{\partial \sigma_0}{\partial pH_s} = -e \frac{\partial [B]}{\partial pH_s}$$

where $pH_s$ is the surface pH value close to the sensor surface. This further yields

$$\frac{\partial \sigma_0}{\partial pH_s} = -eN_s \frac{K_b [H^+]^2 + 4K_a K_b [H^+] + K_a K_b^2}{(K_a K_b + K_b [H^+] + [H^+]^2)^2} - 2.3[H^+] = -e\beta_{int}$$
where $\beta_{\text{int}}$ is the intrinsic buffer capacity. To find a relationship between the surface potential $\psi_0$ and surface $pH_s$ value, one has to find dependence of surface potential on surface charge. To achieve this one can assume the Gouy-Caupman-Stern model which was previously explained (van Hal et al., 1995). This model assumes that the diffusive charge (double-layer charge) extends beyond the outer Helmholtz plane. Since the double layer capacitance is very large compared to the nanowire capacitance one can assume $\sigma_{DL} \approx -\sigma_0$ where $\sigma_{DL}$ can be calculated using Poisson-Boltzmann equation for semiconductor-electrolyte interface:

$$\sigma_{DL} = -(8k_B T \varepsilon_W \varepsilon_0 c_0)^{1/2} \sinh \left( \frac{z e \phi}{2k_B T} \right) = -\sigma_0$$

where $\phi$ is the potential at the outer Helmholtz plane. This further yields:

$$\frac{\partial \psi_0}{\partial pH_s} = \frac{\partial \psi_0}{\partial \sigma_0} \frac{\partial \sigma_0}{\partial pH_s} = -e \frac{\beta_{\text{int}}}{C_{\text{dif}}}$$

This gives a general expression for the change of surface potential with the change of $pH$ value at the sensor surface (van Hal et al., 1995):

$$\frac{\partial \psi_0}{\partial pH_B} = -2.3 \frac{k_B T}{e} \alpha$$

The parameter $\alpha$ represents the sensitivity parameter which describes the responsivity of the sensor surface and is defined by $\alpha = \frac{1}{\left( \frac{2.3 k_B T C_{\text{dif}}}{e \beta_{\text{int}}} \right) + 1}$. The maximum possible value for the sensitivity of the bioFET is defined as Nernstian response (when $\alpha = 1$) and is approximately $60 \frac{mV}{pH}$, a value that is analogue to the subtreshold swing value of $60 \frac{mV}{\text{dec}}$. The maximum possible
response for a ion sensitive sensors are therefore limited to $1^{\text{dec}}_{\text{pH}}$, when operated in subthreshold regime.

### 2.3 BioFET Response scaling

BioFET scaling has been the topic of long discussions among the bioFET community (Nair and Alam, 2007). In order to explore the relationship between the magnitude of device response and the size of the nanowire we develop a simple model for a bioFET in an electrolyte solution. Figure 2.6 depicts the model of semiconductor-insulator-electrolyte-reference electrode system. We assume that the active silicon layer has a thickness of $t_{\text{Si}}$ and the insulator layer is $t_{\text{ox}}$. To describe the electrolyte we used the Debye-Huckel approximation (Israelachvili, 1991) for a single valence two-component electrolyte.

![Figure 2.6 Simplified model of a nanoribbon bioFET excluding the edge effect of the side walls.](image)
The basic principle behind label-free sensing is the following – the change of surface charge $\sigma_s$ induces charges in both semiconductor and electrolyte i.e. $\sigma_0 = -(\sigma_{sc} + \sigma_{DL})$. To describe this system one can write a system of Poisson-Boltzman equations (Sorensen et al., 2007):

$$\frac{d^2\phi(z)}{dz^2} = \begin{cases} \lambda_{D,si}^{-2}\phi(z), & 0 < z < t_{si} \\ 0, & t_{si} < z < t_{si} + t_{ox} \\ \lambda_D^{-2}\phi(r), & z > t_{si} + t_{ox} \end{cases}$$

where $\lambda_{D,si}$ is the Debye (Thomas-Fermi) screening length in the semiconductor and $\lambda_D$ is the Debye screening length in the electrolyte. For simplicity we assume that oxide layer is free from charges.

Potential $\phi(z)$ needs to satisfy the following boundary condition at interface:

$$\epsilon_1 \frac{d\phi(z^-)}{dz} - \epsilon_2 \frac{d\phi(z^+)}{dz} = \sigma$$

where $\sigma$ represents free surface charge at the boundary of two media. Since we assume that there are no free charges in the semiconductor and insulator, the potential $\phi$ is continuous at their boundary. The only discontinuity is expected at the interface of the insulator and electrolyte as shown in Figure 2.7.
The change of the surface potential $\Delta \sigma_0$ will cause a change in the semiconductor carrier concentration of $\Delta n$. Therefore the total change in conductance and therefore current will be given by $\Delta G = e\mu \Delta n$. The sensitivity of the sensor is (Janata, 2009):

$$S = \frac{\Delta G}{G_0} = \frac{\epsilon_{Si}}{\lambda_{D,\text{Si}}^2 en_0} \frac{\int dr \phi(r)}{\int dr}$$

Figure 2.8 Device sensitivity as a function of thickness of the silicon active layer for two different values of Debye screening length, $\lambda_{TF}$, in Silicon.
The change in bioFET conductance \( \Delta G \) is directly related to the magnitude of induced charge in the semiconductor due to the binding of charges at the surface, Figure 2.7. The magnitude of the induced charge will greatly depend on the doping of the semiconductor which determines the spatial extent of the surface charge effects. This value is directly related to the Debye screening length in the semiconductor.

Using the formula for sensitivity and by numerically solving Poisson-Boltzmann differential equation for bioFET we obtain sensitivity dependence \( S \) on device thickness for two different Debye Screening lengths in silicon. Figure 2.8 shows dependence of the sensitivity as a function of silicon thickness. Two values of Debye length are considered – one of 3nm which corresponds to a high doping and one of 20 nm corresponding to a lower doping densities. As it can be seen, in case of strong screening effect we observe well known inverse scaling law \( \left( \frac{1}{t_{Si}} \right) \) (Nair and Alam, 2007). In case of lower doping i.e. weaker screening in the semiconductor more of the surface charge is reflected in the semiconductor and in the same time there is an optimal thickness where the sensitivity reaches maximum value.

![Figure 2.9 The spatial extent of the surface charge effect inside the bioFET in case of different silicon active layer thicknesses.](image)

Observed effects can be explained using a simple approach demonstrated in Figure 2.9. The binding of the surface charge to the sensor surface causes a change in the semiconductor that can
be “felt” typically at $\sim \lambda_{D, Si}$ inside the semiconductor. In case of a thicker material this effect
does not utilize the full body of the semiconductor and sensitivity $S = \frac{\Delta G}{G_0}$ is not maximized
unlike in the case when the silicon thickness is on the order of $\lambda_{D, Si}$. This picture, although
simplified, agrees with the more complex numerical models (Baumgartner et al., 2011).

Similar dependency can be expected for width scaling, however if the Debye length is on the
order of the silicon thickness (which is true in our case) one could expect discrepancy for larger
values of widths. In case the thickness is on the same order of the Debye length the effect of the
surface charge reaches maximum, however the effect of the side-walls will become more
prominent when the width becomes on the order of $2-3 \lambda_{D, Si}$, and the influence of two side
surfaces start overlapping, Figure 2.10.

![Figure 2.10 Sensitivity scaling as a function of device width and predicted deviation of the model at larger widths.](image)
2.4 Relationship between electrical characteristics and sensor response

To understand this relationship one has to go back to the interaction between the analyte molecules and the sensor surface. We start with the simple 1:1 receptor-ligand binding kinetics described by the following first-order kinetic equation (Sadana, 2003):

\[ \frac{dR}{dt} = k_{on} c (1 - R) - k_{off} R \]

where \( R \) is the relative amount of bound analyte, \( c \) is the analyte concentration at the sensor surface, and \( k_{on} \) and \( k_{off} \) are the association and dissociation rate constants, respectively. The kinetic equation leads to the following expression for the binding site occupancy versus time (Homola, 2006):

\[ R(t) = \left[ \frac{k_{on} c}{k_{on} c + k_{off}} - R_0 \right] \left( 1 - e^{-(k_{on} c + k_{off})t} \right) + R_0 \]

where \( R_0 \) is the relative number of the initially occupied binding sites at \( t = 0 \).

BioFETs, as any other type of sensors, is typically utilized for end-point detection i.e. upon addition of the analyte there is a detection time during which device signal reaches saturation level for a given analyte concentration. However, there is little in the literature quantifying the correlation between device response, its electrical characteristics and analyte concentration. Starting from the equation for ISFET current in the linear region for \( V_{DS} \ll V_{GS} - V_T \):

\[ i_{ds} = k \cdot (V_{GS} - V_T) V_{DS} \]
where $V_T$ was previously defined. Upon binding of molecules to the sensor surface the change of the surface potential $\Delta \psi_0$ causes a shift in the device threshold voltage and thus a change in sensor current:

$$d i_{ds} = -kV_{DS} = -kV_{DS}dV_T$$

which yields

$$\Delta i_{ds} = -g_m \Delta \psi_0$$

since $g_m = \frac{\partial i_{ds}}{\partial V_{GS}} = kV_{DS}$. However some of the binding events take longer time to stabilize in case of the lower analyte concentrations, lower association and higher dissociation rates. The binding rate just after analyte addition ($t \rightarrow 0^+$):

$$\dot{R}(t \rightarrow 0^+) = k_{on}c - R_0(k_{on}c + k_{off})$$

In case of an unused sensor i.e. $R_0 = 0$ the initial binding rate is $\dot{R}(t \rightarrow 0^+) = k_{on}c$ is proportional to the analyte concentration. Furthermore, if the change of the surface charge is not high which at $t \ll T_{eq}$, where $T_{eq}$ is the time needed for surface binding to reach equilibrium, is true, the change of the surface potential $\psi_0$ and therefore device current $i_{ds}$ is linearly proportional to the change of surface charge $\delta \sigma_0$. This can be shown by assuming $\sigma_0 \approx -\frac{2 \epsilon_W k_B T}{\lambda_D} \sinh \left( \frac{\psi_0}{2k_B T} \right)$ which for small changes of surface potential yields $\sigma_0 \approx -\frac{2 \epsilon_W k_B T}{\lambda_D} \frac{\psi_0}{2k_B T} = -\frac{\epsilon_W}{\lambda_D} \psi_0$ and $\delta \sigma_0 \sim \delta \psi_0$.

This means that the initial current rates are directly proportional to the concentration of analyte and can be employed for quantification of analytes. *This is a novel approach that solves the quantification problem, as all prior nanowire/nanoFET detection work utilized endpoint*
detection, and struggled with calibration issues. Furthermore, the initial binding rate measurement requires shorter time than the endpoint detection which in very low concentration cases can take thousands of seconds. The dependence of sensor response on its electrical characteristics such as baseline current and transconductance can be used to suppress device-to-device variation and to allow internal calibration of the ISFET based sensors.

2.5 BioFET response in the presence of Debye Screening

To fully understand the bioFET behavior in the presence of electrolyte screening and the effects of the screening on sensor signal we developed electrostatic model based on the Poisson-Boltzmann equation for semiconductor-electrolyte system.

Starting from general Poisson-Boltzmann equation (Windbacher et al., 2009):

\[
\nabla \cdot [\varepsilon_0 \varepsilon(r) \nabla \phi(r)] = -\rho^f(r) - \sum_i c_i z_i q \exp \left[ \frac{-z_i q \phi(r)}{k_B T} \right]
\]

where \( \varepsilon(r) \) is dielectric constant of the electrolyte, \( \rho^f(r) \) is density of free electric charge, \( z_i \) is the valence of \( i^{th} \) ionic species and \( c_i \) is its concentration in bulk of the solution. Assuming no free charge in the solution and uniform dielectric constant one obtains:

\[
\varepsilon_0 \varepsilon \nabla^2 \phi(r) = -\sum_i c_i z_i q \exp \left[ \frac{-z_i q \phi(r)}{k_B T} \right]
\]

Assuming only a two-component electrolyte with single valence ions yields:

\[
\varepsilon_0 \varepsilon \nabla^2 \phi(r) = c_0 q \left[ \exp \left( \frac{q \phi(r)}{k_B T} \right) - \exp \left( -\frac{q \phi(r)}{k_B T} \right) \right].
\]
The final form of the Poisson-Boltzmann equation for two-component electrolyte can be written as:

\[ \nabla^2 \phi(r) = \frac{2c_0q}{\epsilon_0\epsilon} \sinh \left( \frac{q\phi(r)}{k_B T} \right) \]

Recall the operation of an ISFET (bioFET) in the linear region is given by the following equation (Bergveld, 1981)

\[
I_D = \mu C_{ox} \frac{W}{L} \left\{ [V_{gs} - (E_{ref} - \psi_0 + \chi^{sol} - \frac{\Phi_{Si}}{q} - \frac{Q_{ox} + Q_{ss}}{C_{ox}} - \frac{Q_B}{C_{ox}} + 2\phi_f)]V_{ds} - \frac{1}{2}V_{ds}^2 \right\}
\]

which can be further approximated as:

\[
I_D = \mu C_{ox} \frac{W}{L} [V_{gs} - V_T + \psi_0]V_{ds}
\]

where all parameters have been previously defined. All these parameters are independent on pH changes except for the electrostatic surface potential \( \psi_0 \). This potential drop between the semiconductor and insulator surface represents the chemical and biological processes at the insulator-electrolyte interface and every biological or chemical reaction that occurs here will be reflected in its modification. Assuming a constant bias, a modulation in the potential drop \( \psi_0 \) will cause a direct change of both oxide and semiconductor potentials, \( \psi_{ox} \) and \( \psi_s \). Assuming surface sheet charge \( \sigma_0 \) at the bioFET surface as shown in Figure 2.11 one can calculate the corresponding induced charge in the solution and the semiconductor by solving Poisson-Boltzmann equation.
\[
\frac{d^2 \psi_0}{dz^2} = \frac{2qc_0}{\varepsilon_0 \varepsilon_W} \sinh \left( \frac{q \psi_0}{k_B T} \right).
\]

Substituting the expression for the Debye length \( \lambda_D = \sqrt{\frac{k_B T \varepsilon_0 \varepsilon_W}{2q^2 c_0}} \) leads to the following equation:

\[
\frac{d^2 \psi_0}{dz^2} = \frac{k_B T}{q \lambda_D^2} \sinh \left( \frac{q \psi_0}{k_B T} \right).
\]

Knowing that \( \frac{d}{dz} \left( \psi_0' \right)^2 = \frac{d}{dz} \left( \frac{d \psi_0}{dz} \right)^2 = 2 \frac{d \psi_0^2}{dz^2} \frac{d \psi_0}{dz} \) into the previous equation yields

\[
\frac{1}{2} \frac{d}{\psi_0 dz} \left( \psi_0' \right)^2 = \frac{k_B T}{q \lambda_D^2} \sinh \left( \frac{q \psi_0}{k_B T} \right)
\]

or further

\[
\frac{d}{dz} \left( \psi_0' \right)^2 = \frac{2k_B T}{q \lambda_D^2} \sinh \left( \frac{q \psi_0}{k_B T} \right).
\]

By direct integration one can obtain the following expression:

\[
\left( \psi_0' \right)^2 = \frac{2k_B T}{q \lambda_D^2} \int_{-\infty}^{\psi_0} \psi_0' \sinh \left( \frac{q \psi_0}{k_B T} \right) dz = \frac{2(k_B T)^2}{q^2 \lambda_D^2} \left( \cosh \left( \frac{q \psi_0}{k_B T} \right) - 1 \right) = \frac{4(k_B T)^2 \sinh \left( \frac{q \psi_0}{2k_B T} \right)}{\lambda_D^2 q^2},
\]

this yields

\[
\psi_0' = \frac{\pm 2k_B T \sinh \left( \frac{q \psi_0}{2k_B T} \right)}{\lambda_D q}.
\]
Applying Gauss’ law for the surface charge $\sigma_0 = D \cdot n = -\epsilon_0 \epsilon W \nabla \psi_0 \cdot n = -\epsilon_0 \epsilon W \psi'_0$ which yields the final expression for the total charge in the Gouy-Chapman layer (Verwey and Overbeek, 1999):

$$\sigma_{DL} = -\sigma_0 = \mp \sqrt{8 \epsilon_0 \epsilon W k_B T c_0} \sinh \left( \frac{q \psi_0}{2 k_B T} \right).$$

In order to quantify the effect of the buffer ionic strength and surface molecule configuration to device signal, one needs to develop a sensing model for a nanowire in presence of Debye screening.

To understand the effect of (solution) Debye screening on a sensor signal we develop a simple model starting from a cylindrical silicon nanowire with radius $R$, surface oxide thickness of $\delta$ and surface charge $\sigma_s$ at distance $l$ from nanowire surface, Figure 2.12. The initial nanowire conductance $G_0$ and carrier density $n_0$ is modulated by $\Delta G_0$ and $\Delta n_0$, respectively. In order to solve this problem one can use dilute electrolyte model (Debye-Hückel approximation, (Debye
and Huckel, 1923) and semiconductor Thomas-Fermi model in conjunction with the Poisson equation.

Figure 2.12 Model used for solving Poisson equation for nanowire in an electrolyte solution surrounded by charge sheet of $\sigma_s$

The linear differential equation for the induced electrical potential $\phi$ is:

$$\nabla^2 \phi(r) = \begin{cases} 
    \lambda_{TF}^2 \phi(r), & r \in \Omega_1 \\
    0, & r \in \Omega_2 \\
    \lambda_D^{-2} \phi(r), & r \in \Omega_3 \cup \Omega_4 
\end{cases},$$

where $\lambda_{TF}$ is the Thomas-Fermi screening length in the nanowire ($\Omega_1 = \{r | 0 < |r| < R\}$) and $\lambda_D$ is the Debye screening length in electrolyte ($\Omega_3 = \{r | R + \delta < |r| < l\}, \Omega_4 = \{r | |r| > l\}$). The nanowire insulating layer (typically an oxide) is represented by domain $\Omega_2$ ($\Omega_2 = \{r | R < |r| < R + \delta\}$), and is assumed to be free from charges. In addition potential $\phi$ needs to satisfy the following boundary conditions at each of the interfaces (Jackson, 2007):
\[
\phi_i(r) - \phi_{i+1}(r) = 0 \\
n \cdot \nabla (\varepsilon_i \phi_i(r) - \varepsilon_{i+1} \phi_{i+1}(r)) = \sigma_{i,i+1}
\]

for \( r \in \partial \Omega_{i,i+1} \), where \( \partial \Omega_{i,i+1} \) is a boundary surface separating domains \( \Omega_i \) and \( \Omega_{i+1} \). For simplicity one can assume the following \( \sigma_{1,2} = \sigma_{2,3} = \sigma_5, \varepsilon_1 = \varepsilon_{Si}, \varepsilon_2 = \varepsilon_{ox} \) and \( \varepsilon_3 = \varepsilon_4 = \varepsilon_w \).

The induced charge \( \Delta n \) and the change in conductance \( \Delta G = L^{-1} \int_{\Omega_i} e\Delta n(r) \mu d^3r \) of the nanowire due to the surface charge \( \sigma_S \) can be found by solving Poisson equation:

\[
-e\Delta n(r) = -\varepsilon_1 \nabla^2 \phi_1(r) = -\frac{\varepsilon_1}{\lambda_{TF}^2} \phi_1(r).
\]

This system can be solved for oxide thickness \( \delta \ll R \), (Sorensen et al., 2007) to obtain

\[
\frac{\Delta G}{G} \sim \Gamma_i \sigma_l
\]

where \( \Gamma \) represents device sensitivity on surface charge while \( \Gamma_l \) is the Debye screening factor (De Vico et al., 2011). The sensitivity factor \( \Gamma \) can be approximated as:

\[
\Gamma \approx \left[ 1 + \frac{I_0 \left( \frac{R}{\lambda_{TF}} \right) K_1 \left( \frac{R}{\lambda_D} \right)}{I_1 \left( \frac{R}{\lambda_{TF}} \right) K_0 \left( \frac{R}{\lambda_D} \right)} \right]^{-1}
\]

which asymptotically approaches unity and is slow varying for large \( R/\lambda_D \) (which is true in case of a planar sensor) as it can been seen from Figure 2.13. The Debye screening factor \( \Gamma_l \) is given by (De Vico et al., 2011):

\[
\Gamma_l = 2 \frac{R}{R + l} \left[ 1 + \sqrt{\frac{R}{R + l}} \exp \left( \frac{l}{\lambda_D} \right) \right]^{-1}.
\]
To transition to a planar (nanoribbon) sensor we let $R \to \infty$ in the previous equation which yields:

$$\Gamma_i \approx 2 \left[ 1 + \exp \left( \frac{l_{\lambda_D}}{\lambda_D} \right) \right]^{-1}.$$ 

The change of bioFET current due to binding of charged molecules under buffer conditions that has solution Debye screening length $\lambda_D$ is therefore:

$$\frac{\Delta I}{I_0} = \frac{I(\lambda_D) - I_0}{I_0} = \Gamma_i \sigma_S$$

The variable $I_0 \Gamma \sigma_S$ represents a change in sensor current in absence of debye screening i.e. $\lambda_D \to \infty$ and $\Gamma_i \to 1$. This yields

$$\frac{\Delta I}{\Delta I_{\text{max}}} = \frac{I(\lambda_D) - I_0}{\Delta I_{\text{max}}} = \Gamma_i = 2 \left[ 1 + \exp \left( \frac{l_{\lambda_D}}{\lambda_D} \right) \right]^{-1}.$$ 

Using previous equation and measurements of device current at different Debye lengths one can extract the average spatial extent of bound molecules at the nanosensor surface.

![Figure 2.13 Dependence of a nanowire sensitivity factor $\Gamma(z)$ on $z = R/\lambda_D$. In the operating region this function is slow varying and is close to 0.5](image)
The bioFET current change $\Delta I$ can be theoretically estimated using its solution transconductance $g_m$ and the change of its surface potential due to charge binding. To get to the approximate dependence one can apply a simplified capacitance model for the system.

Based on the previously detailed model of a bioFET-electrolyte system (beginning of this section) one can assume an idealized capacitor model shown in Figure 2.14.

![Capacitor model for bioFET in electrolyte solution.](image)

Assuming that potential of the reference electrode with respect to the ground is $v_g$ one can calculate the surface potential $\psi_0$ at the bioFET-electrolyte interface using voltage divider:

$$
\psi_0 = \frac{C_{dl}}{C_{dl} + C_{fet}} v_g
$$

where $C_{dl}$ consists of Gouy-Chapman and Stern/Helmholtz layer capacitances in series, and $C_{fet}$ consists of $C_{nw}$ and $C_{ox}$, in series. The change of device current $\Delta i$ due to the change of surface potential $\Delta \psi_0$ is given by:

$$
\Delta i = \frac{\partial i}{\partial \psi_0} \Delta \psi_0.
$$

Using capacitor voltage divider from Figure 2.14 one can write:

$$
\frac{\partial \psi_0}{\partial v_g} = \frac{C_{dl}}{C_{dl} + C_{fet}}.
$$
The current change can therefore be expressed as:

$$\Delta i = \frac{\partial i}{\partial v_{g}} \frac{\partial v_{g}}{\partial \psi_{0}} \Delta \psi_{0} = \left(1 + \frac{C_{fet}}{C_{dl}}\right) g_{m} \Delta \psi_{0},$$

where $C_{fet} = C_{dl} \parallel C_{ox} = \left(\frac{\varepsilon_{0} \varepsilon_{Si}}{x_{d}} A\right) \parallel \left(\frac{\varepsilon_{0} \varepsilon_{ox}}{t_{ox}} A\right) \approx \frac{\varepsilon_{0} \varepsilon_{Si}}{\lambda_{D}} A$ and $C_{dl} \approx \frac{\varepsilon_{0} \varepsilon_{W}}{\lambda_{D}} A$ which yields

$$\Delta i = \left(1 + \frac{\varepsilon_{Si} \lambda_{D}}{\varepsilon_{W} x_{d}}\right) g_{m} \Delta \psi_{0} \approx g_{m} \Delta \psi_{0},$$

since $\frac{\varepsilon_{Si} \lambda_{D}}{\varepsilon_{W} x_{d}} \approx 10^{10} \text{nm} < 1$. A similar relationships between the capacitances have been obtained using more precise models (Georgiou and Toumazou, 2009), $C_{dl} = 462pF \gg C_{ox} = 427pF > C_{d} = C_{nw} = 150pF$.

### 2.6 Summary

In this chapter presented are several theoretical approaches and models for nanoscale bioFET in electrolyte environments with main purpose of establishing connection between the bioFET electrical response and its electrical characteristics as well as the properties of electrolyte and sensed molecules. Using Poisson-Boltzmann equation for semiconductor-oxide-electrolyte system we explain the effect surface charge on sensor response as function of device geometrical and electrical parameters. We found that Debye screening in the semiconductor plays a major role in the spatial extent of the effect of the bound charges to the nanosensor signal. This effect greatly depends on the ratio of geometrical and electrical thickness of the charge carriers in the semiconductor. The closer the geometrical size to the Debye length is the higher the sensor response. This value reaches optimal value for thicknesses that are on the order of device thickness.
Using elementary binding kinetics and establishing the relationship between the amount of bound charge and surface potential of the bioFET we theoretically show that initial kinetic rates measured by bioFET are directly proportional to changes in the surface potential and surface charge therefore establishing a different way of quantifying analyte concentration using field effect sensors, in addition to commonly used end-point detection. Moreover, we demonstrate that sensor response is directly related to device transconductance which leads to a method of nanosensor internal calibration and their application as quantitative rather than just qualitative tools. This approach is further developed in Chapter 4.

Similarly, using Poisson-Boltzmann equation for nanosensor-electrolyte system we were able to quantify the effect of Debye screening in the electrolyte on device signal. By changing the Debye length of the sensing buffer one can explore spatial extent of the bound charge. This approach paves the way for the Debye length modulation technique which is discussed in Chapter 5.
3. BioFET fabrication and characterization

3.1 Introduction
Over the past years several different hybrid and bottom-up methods for nanoscale bioFET fabrication have been demonstrated (Quitoriano and Kamins, 2008, McAlpine et al., 2007). However, two issues are seen as major drawbacks of incorporating these methods in mass production techniques. First and foremost, the use of CVD grown nanowires is incompatible with mainstream CMOS technologies which makes integration with on-chip amplifiers and data processing circuits practically impossible. The inability to integrate bottom-up nanowire FET with microelectronic components and microfluidics directly hinders application for multiplexed detection schemes and assays. Finally, the low yield and inability to produce large number of devices with similar electrical characteristics (Stern et al., 2005) prevents analyte quantification which is critical for a competitive point-of-care (POC) diagnostic tool.

Presented here, are two CMOS-compatible fabrication processes for nanoscale bioFETs. The first process was developed for nanoribbon bioFETs (Vacic et al., 2011) which are in essence two dimensional sensors with nanometer scale thickness (20-30nm) and micron size lateral dimensions. These sheets of silicon (known as mesas) are carved from active silicon layer using dry or wet etching processes (Elfstrom et al., 2008). The rest of the processing is done in the same manner as a traditional MOSFET which includes source/drain doping, oxide growth and metallization (Plummer).

The second process was developed for both nanoribbons and nanowires with the ability to multiplex devices of all sizes on a same chip. Moreover, instead of a native oxide which was
used as an insulator layer in the first series of bioFETs we introduced possibility of using dry thermal oxide, Al₂O₃, HfO₂ or Ta₂O₅.

### 3.2 Series A fabrication process- nanoribbon bioFETs

Eight inch silicon-on-insulator wafers with 70 nm active and 145 nm buried oxide (BOX) layer were purchased from SOITEC. In Figure 3.1-i, the active silicon layer is shown in pink, the BOX in blue, and the silicon substrate (handle) in gray. The doping in the active and handle wafers was boron at 10¹⁵ cm⁻³. The wafers were laser-cut to 4-inch diameters by Silicon Quest International. Processing was performed in part at the Cornell Nanoscale Science and Technology Facility and the Yale Center for Microelectronic Materials and Structures. All photolithography steps were performed using Shipley S1808, S1813, or S1827 photoresist and an EV Group 620 mask aligner. All masks were 5” and were purchased from PhotoSciences, Inc.

The active layer was thinned to 25-50 nm (depending on the intended application) by thermal growth of oxide at 1100°C using an MRL Industries furnace after MOS cleaning, Figure 3.1-ii. The oxide thickness was determined using a Woollam Variable Angle Spectroscopic Ellipsometer.

As shown in Figure 3.1-iii the active parts of the sensor (mesas) were patterned in the first lithography step followed by a chlorine reactive-ion etching (RIE, Oxford PlasmaLab 100).
Figure 3.1 Fabrication process of top-down silicon bioFETs using only optical lithography.
Chlorine chemistry was used because it does not etch silicon oxide, thus the BOX serves as an etch-stop. Photoresist was stripped by ozone plasma using a Mercator Control System Inc. HF-6 barrel asher.

The second PL step (Figure 3.1-iv) is used to pattern contacts to the silicon handle wafer which serves as an electronic backgates for device characterization. Vias through the BOX to the backgate were etched using 10:1 buffered oxide etch (BrandNu Labs) and photoresist was stripped using acetone and isopropanol (BrandNu Labs).

The third PL step (Figure 3.1-v) patterned degenerate doping regions for contacts to device and backgate contacts. A Boron implant dose of $5 \times 10^{15}$ cm$^{-2}$ at 8 KeV was performed at a $7^\circ$ tilt by Core Systems. In Figure 3.1-v, the doped regions are shown as graded pattern. Photoresist was then stripped by ashing, followed by wafer exposure to piranha. The dopant was activated by annealing the wafers at 900°C in nitrogen in a MRL Industries furnace after MOS cleaning.

The fourth PL step (Figure 3.1-vi) patterned metal leads, pads, and contacts. A 145 nm Al (99.99%, Kurt J. Lesker Co.) / 5 nm TiW (90/10, w/w, Kurt J. Lesker Co.) liftoff evaporation was performed by electron-beam deposition in a Kurt J. Lesker EJ1800 Thin Film Deposition System. After liftoff, achieved by wafer sonication in acetone, the wafers were rapid-thermal annealed (RTA) for 1 min at 450°C in a Surface Sciences Integration Solaris 150 RTA. Sequential RTA / electrical characterization steps dictated that these conditions were required in order to form Ohmic contacts to devices. In Figure 3.1-vi, the metal layer is shown in green.

The fifth PL step (Figure 3.1-vii) patterned S1808 photoresist as a passivating layer across the chip to prevent leakage. Exposed surfaces included contacts and active device regions. The photoresist was hard baked for 1 hr at 140 °C. Note that this step was performed after 3-
aminopropyltriethoxysilane (APTES) functionalization as resist is dissolved by the organic solvents required for that process. In such case the whole wafer was immersed in the 20% (v/v) toluene solution of APTES. Optical and Scanning Electron (SEM) micrographs of completed devices are shown in the inset in Figure 3.2.

![Optical and SEM micrographs of bioFETs. The arrow points to the sensing area of the device exposed to solution.](image)

**Figure 3.2** Optical and SEM micrographs of bioFETs. The arrow points to the sensing area of the device exposed to solution.

### 3.3 Series AbioFET electrical characterization

Upon fabrication but before sensing experiments it is important to explore the quality of fabricated devices. Typical methods are similar to the ones used for MOSFETs and include I-V characteristics, threshold voltage distribution and mobility extraction. These parameters carry important information about the uniformity and quality of fabrication process. Without a good
control over device parameters and due to device-to-device variation, the application of bioFETs as a quantitative sensor is practically impossible.

In order to understand how device response is related to its electrical characteristics it is necessary to characterize device prior to and after the functionalization. Device electrical characteristics were assessed by simultaneous measurement of $I_{DS}$-$V_{GS}$ characteristics of up to 8 devices on a given die. To understand the uniformity of our fabrication process we investigated the variation of the threshold voltage as a function of its position on the wafer. For a group of 106 devices used for this experiment we obtained the average for threshold voltage $<V_T> = -2.3V$. The standard deviation for the sample was calculated to be $\sigma_{V_T} = 0.15V$ and the standard error of the mean $\sigma_{<V_T>} = 0.014V$.

![Figure 3.3 Threshold voltage distribution of nanoribbon devices across a 4” wafer. Each data point is a 5x5 mm die containing up to 8 devices. The inset shows approximate die positions on the wafer. Notation a) and b) in the die label refers to the lower and the upper half of a 10 x 5 mm die. (10,3) is a center column die.](image)

The distribution of threshold voltages is dominated by the nonuniform thickness of the active silicon layer ($70\pm10(3\sigma)$nm) and variations in the thickness of the dry oxide used for thinning of
active region (estimated to be around 7-8% across the 4” wafer). Figure 3.3 shows the variations of threshold voltage for a series of dies, and the corresponding wafer map (inset). In regions with minimal thickness fluctuations (wafer center), we obtained threshold voltage variation of 8mV, in terms of standard error of the mean.

The off current of dry devices is measured to be on the order of 1fA to 100fA which yields good on/off ratio of approximately 5-6 orders of magnitude. The average backgated subthreshold swing is (630±30) mV/dec which corresponds to the thickness of 145nm of buried oxide layer between the active and the handle (backgate). The average drift mobility obtained from peak transconductance is calculated to be (93±17) cm²/Vs which is in agreement with previously obtained values measured on fully depleted SOI RIE defined devices (Stern et al., 2007a, Sun and Plummer, 1980, Habicht et al., 2010).

Figure 3.4 shows I-V characteristics for 8 devices on the same chip. As expected from a p-type accumulation mode device, it exhibits negative threshold voltages, $V_T = (-2.43 \pm 0.09)V$. The average subthreshold swing is calculated to be 700 mV/dec which is in agreement with the backgate oxide thickness of 145nm. The relation between the subthreshold swing and oxide capacitance $C_{ox}$ is given by (Muller et al., 2003):

$$S = 2.3 \frac{kT}{q} \frac{(C_{ox} + C_{it} + C_d)}{C_{ox}}$$
Figure 3.4 I-V characteristics of 8 devices on a same chip scanned using a multiplexing system. Device sizes are purposely varied to explore the scaling effects. The floor for leakage current (1-10pA) is limited by the leakage current of operational amplifiers used in the transresistance amplifiers.

where \( C_{it} \) is an interface trap capacitance, \( C_d \) is a capacitance of the depletion region, \( k_B \) is the Boltzman constant and \( T \) is absolute temperature. Assuming that there are no interface traps (for rough estimate only) the lower boundary value for the subtreshold swing is then:

\[
S = 2.3 \frac{k_B T}{q} \left( 1 + \frac{C_d}{C_{ox}} \right) \approx 60 \frac{mV}{dec} \left( 1 + \frac{t_{ox}}{x_d} \frac{\varepsilon_{si}}{\varepsilon_{ox}} \right) = 580 \frac{mV}{dec}
\]

Figure 3.5 Transconductance of bioFETs as a function of backgate voltage.
Another parameter that describes the quality of bioFETs is the transconductance $g_m$ and carrier mobility $\mu$. One can extract the value of the field effect mobility from the peak transconductance. Figure 3.5 shows gate voltage $V_G$ dependence of transconductance $g_m$ for devices having various widths and fixed length of 12 $\mu$m. Using the relation between the transconductance and mobility

$$\mu_{FE} = \frac{L}{W} \frac{g_m}{C_{ox} V_{ds}}$$

One estimates the average mobility for bioFETs to be $(80\pm20)$ cm$^2$V$^{-1}$s$^{-1}$ which is in good agreement with the other work on p-type SOI RIE-defined devices (Habicht et al., 2010).

### 3.4 Series B fabrication process – Nanoribbon/Nanowire BioFET

The second generation of bioFETs uses several additional steps in the fabrication process in order to improve overall device quality and allow electron beam lithography as well as on chip pseudo-reference electrode. One of the major shortcomings of the first generation bioFETs was the insulator, native oxide, used as the sensing surface. Due to the high porosity of native oxide the life-time of bioFETs was relatively short and varied from 15min to 2 hours. Some of the Series A devices have shown significant drift and/or leakage current in solutions (Jamash et al., 1998).

In addition to changes in fabrication process, the overall layout of the chips was changed to accommodate more devices for multiplexed sensing and microfluidic integration. Three die sizes were designed: the 3.3 mm by 3.3mm (16 devices total), 6.6 mm by 6.6 mm (32 devices), and 10
mm by 10 mm (32 devices), Figure 3.6. The latter two have staggered devices (4 columns each having 8 devices) which will allow for simultaneous sensing of up to four different biomarkers.

Figure 3.6 Layout of Series B bioFETs illustrating three different die sizes and chip layouts. The die sizes from left to right are 3.3x3.3, 6.6x6.6 and 10x10mm.

The active silicon layer of the SOI wafers was thinned down using thermal oxidation as previously described, Figure 3.7-i and ii.

In the 1st PL step alignment marks were patterned and etched using SF₆/C₄F₈ chemistry, Figure 3.7-iii. This allows both optical and electron-beam lithographic definition of mesas. Shipley 1827 photoresist was used as a masked. A 5-minute etch step yielded 2µm deep alignment marks. This is sufficient enough to achieve good contrast in the electron beam writer (Vistec 5000+) and maintain the overall edge quality of both optical and e-beam alignment marks. The etch process was in-house designed to imitate the standard Bosch process for deep RIE etching of silicon – SF₆ is utilized for etching of silicon (300 nm/min) and silicon oxide (50 nm/min), while C₄F₈ serves for passivation layer formation by forming a polymer on the surface and thus preventing the sidewall etch (Abdolvand and Ayazi, 2008).

The second PL step includes backgate via etch using BOE, Figure 3.7-iv, followed by the third PL step to form implantation regions, Figure 3.7-v. In this process we used BF₂⁺ at 8 keV as a
dopant instead of boron (Sakurai et al., 2006). The heavier BF$_2^+$ has lower range and is used for shallow implantation. The dopant is then activated using rapid thermal annealing (RTA) at 1000°C for 30 seconds instead of thermal activation to prevent redistribution.

The next step involves mesa definition by either photolithography (smallest feature 1mm) or electron-beam lithography (smallest feature 10nm), Figure 3.7-vi. In case of electron beam lithography hydrogen silsesquioxane (HSQ) based photoresist was used (Henschel et al., 2003). When exposed to the electron beam HSQ converts into amorphous silicon dioxide-like structure and can be used as an etch mask. Its crystallinity is further improved by annealing in O$_2$ atmosphere at 700-800°C (Holzwarth et al., 2007). The wafer is then briefly etched (5 seconds) in Oxford 100 RIE using CF$_4$ chemistry to remove the oxide layer formed during the annealing step. Mesas are then etched using 22% TMAH solution (v/v, H$_2$O in water) at 60°C for 90 seconds (Tabata et al., 1992).

Following the mesa definition step, an insulator deposition step is performed, Figure 3.7-vii. This step is crucial since it serves to prolong device life in electrolyte solution and improve overall electrical characteristics. The insulator formation can be performed in two ways: a) by thermal growth (dry oxide) b) atomic layer deposition (ALD).
Figure 3.7 Series B bioFET fabrication process.
In the fifth PL step contact vias are etched using 10:1 BOE followed by a metal deposition and lift-off, Figure 3.7-viii. Total of 145nm Al/5nm Ti was evaporated. For the lift-off process, LOR5A/S1808 bilayer was used in N-methylpirilidone (NMP).

In the 6th PL step Pt was evaporated and similar lift-off process was performed to form on-chip reference electrode, Figure 3.7-ix.

Finally in the seventh PL step, passivation layer is deposited and patterned, Figure 3.7-x. Two types of passivation layer are used –traditional photoresist and more chemically resistive SU-8 polymer. The latter one allows better compatibility with Polydimethylsiloxane (PDMS) based microfluidics.

![Figure 3.8 Second generation of bioFETs. The geometry of sensors is improved so it allows easy microfluidic integration and multiplexing.](image)

The new layout is compatible with the Microcascade Autoprobe System which allows automated I-V screening, Figure 3.8. In addition several Matlab programs were developed to allow automated data analysis to extract relevant electrical parameters.

To understand the distribution of threshold voltages on a larger scale, we investigated its’ dependence as a function of die position as illustrated in Figure 3.9. For each die, color represents average threshold voltage of up to 13 devices. The variation in threshold voltages is
due to the initial non-uniformity of the active silicon layer (70 ± 10nm) in addition to the wafer scale variation in dry oxidation process which contributes to addition 5% variation. There are total 144 dies measured, with a total of approximately 1100 devices.

Figure 3.9 Threshold voltage distribution on a wafer scale for 144 dies. The threshold voltage for each die represents a mean value for, on average, 6 devices. Dark blue color represents dies that were not measured.

Similarly, Figure 3.10 shows standard deviation of threshold voltage per each chip. As mentioned before each chip represents an average of up to 13 devices.
Figure 3.10 Wafer scale distribution of threshold voltage standard deviation. Each die represents a set of up to 13 devices.

Figure 3.11 shows the distribution of the average die threshold voltage and its standard deviation.

Figure 3.11 a) Threshold voltage distribution of 144 dies with approximately 1100 bioFETs. b) Threshold voltage distribution based on 144 dies.
Figure 3.12 shows I-V characteristics of 10 devices on the same 3.3mm by 3.3 mm chip. The average voltage is calculated to be \( \langle V_T \rangle = -2.22\text{V} \) with the standard deviation of \( \sigma = 0.05\text{V} \) and standard error of the mean \( \sigma_{\langle V_T \rangle} = 0.016\text{V} \).

Figure 3.12 Ids-Vgs characteristics of 8 devices on a same die.

Series B fabrication process allows for electron beam lithography (EBL) defined devices. Instead of using optical photoresist (e.g. S1808) in PL4 step described in Figure 3.7-vi one can use a hydrogen silsesquioxane (HSQ) to pattern the mesas. This way we fabricated devices with widths ranging from 60nm to 2000nm on the same die. Sample scanning electron micrograph is shown in Figure 3.13.

Figure 3.13 Scanning Electron Micrograph of nanowire mesas obtained by TMAH etching using HSQ as an etch mask.
To explore the quality of fabricated devices it is necessary to record the $I_{DS}$-$V_{GS}$ characteristics, as shown in Figure 3.14a. Typical leakage current is measured to be between 10-100fA. More importantly this fabrication process demonstrated good control over device threshold voltages and subthreshold swings, Figure 3.14b.

![Figure 3.14 a) Typical device $I_d$-$V_{gs}$ characteristics. b) size dependence of bioFET threshold voltages and subthreshold swings.](image)

### 3.5 Measurement system

In order to investigate reproducibility and repeatability of sensing experiments it is necessary to enable simultaneous acquisition and measurement of multiple bioFET sensors. This allows for minimizing measurement error as well as elimination of false positive response. To achieve this, a custom-made measurement system and chip bonding scheme were developed that allow device multiplexing. The setup allows multiplexed data acquisition of up to 8 devices and is easily expandable to 64. The measurement system allows for recording of both electrical characteristics and real-time current measurements.

For sensing in biological solutions and due the timescale of binding events the sampling time is typically chosen to be between 100ms and 500ms for each device i.e. 2-10Hz. The maximum
sampling rate if all 8 devices are used for measurement is therefore 125Hz per device (8ms) which can be used for binding kinetics experiments.

![Transresistance converter schematic](image)

**Figure 3.15 a) Transresistance converter is used to interface the bioFET and the DAQ card. b) BioFET array chip packaging.**

The basic element of data acquisition (DAQ) system is a transresistance amplifier (Horowitz and Hill, 1989) shown in schematics in Figure 3.15. A set of 8 low-noise low-leakage current operational amplifier LT1012 is used to make 8 transresistance amplifiers with feedback resistors of 1MΩ. The magnitude of the feedback resistor is chosen in order for the amplified signal to satisfy the input range of the DAQ card which is used to convert the analog into digital data. Typical operating currents of the bioFETs are 10nA to 1µA. The amplification of $10^6 \frac{V}{A}$, translates the desired current range into a voltage range acceptable for the DAQ card (-10V to 10V). Minimal resolution of the DAQ card is 1µV. The bioFET drain is set at the voltage determined by the analog output of the DAQ card and is typically between 0.1V and 1V – this voltage is common for all 8 devices on a chip. Each of the sources is connected to the inverting input of the op-amp while the non-inverting input is kept at a desired potential – usually ground. These two parameters set the drain-source voltage of the device and keep it constant throughout the experiment. The backgate voltage, common for all devices, is set by either the DAQ card (if it is up to ±10V) or a Keithley 2400 (for voltages beyond the -10 to 10V interval). The DAQ card
is controlled using a LabView program. The sampling rate is adjustable with a maximum value of 1000Hz.

The chip is mounted on a 28-pin header (Semiconductor Materials, Inc.) and contacted using a West Bond wirebonder. A custom-made mixing chamber made from Tygon© tubing is mounted on a chip and glued using an epoxy, Figure 3.15.

The schematic of the whole setup is shown in Figure 3.16. Three main components are connector box which allow interface between the chip and the amplifiers, amplifier box which allows current to voltage conversion and DAQ card controlled by a custom-made LabView program which does analog-to-digital conversion.

![Figure 3.16 Multiplexing setup used for recording device electrical characteristics and real-time sensor response.](image)

### 3.6 Solution gating

A solution gate is an important part of a bioFET. It serves as a pseudo-reference electrode which sets a solution potential to a fixed value (Chen et al., 2006). In addition, by recording solution
gate I-V characteristics and measuring the shift in threshold voltage due to pH changes or surface molecule binding one can measure the sensitivity of the sensor surface or the equivalent voltage shift which was discussed in Chapter 2 and is defined by Nernst equation:

$$\frac{\partial \psi_0}{\partial pH_B} = -2.3 \frac{k_B T}{e} \alpha \leq 60 \frac{mV}{pH}$$

By using both solution and back gate one can optimize the sensitivity of the bioFET and thus bias it in the region where it is highest.

To investigate the quality of bioFETs as sensors, solution gating is performed via Ag/AgCl electrode immersed in a solution. Figure 3.17 shows a schematics of a setup used for device gating in an electrolyte solution (1.37mM phosphate buffer saline, PBS) which is typically used as a sensing buffer in electronic label-free experiments. Here we go a step further and investigate gating of a device using both solution and backgate.

Figure 3.17 Schematics of a double gate bioFET setup used for sensitivity optimization.

Figure 3.18 qualitatively describes the effects of solution and backgate voltage on the mode of operation of such device. Typically, bioFET devices made from SOI with active thickness of 20-40 are fully depleted with no bias applied. For operation of devices in inversion and
accumulation modes, one can apply positive or negative bias, respectively, to the solution and backgate (Elibol et al., 2008).

Figure 3.18 Qualitative description of a double gate bioFET operation. Blue circles represent holes.

To analyze the effect of operating conditions on bioFETs we analyzed device electrical characteristics at different pH levels. In the case of a p-type device accumulation mode device, an increase in pH level will cause deprotonation of the surface silanol groups thus causing an increase in negative surface charge which then increases device current. The increase of current is equivalent to a shift in threshold voltage towards less negative values. Figure 3.19 shows device currents at pH 5 for different values of gate voltages. As expected, both solution and backgate contribute to the conductance and once can extract mutual dependence of the threshold voltages. By applying more negative values to the gates one achieves stronger accumulation of holes at the interfaces and thus increases the source-drain current of the bioFET. Using the data from Figure 3.19, one can extract the dependence of solution gate voltage on the back gate voltage.
As shown in Figure 3.20 solution gate threshold voltage is positive, and it drops (shifts right) as the back gate voltage becomes less negative. The direction of change is expected as by decreasing the back gate voltages towards more negative values causes accumulation of holes and therefore lower solution gate voltage to actually turn the device on.

Similarly in Figure 3.20b), the dependence of the backgate threshold voltage has an expected trend i.e. increasing towards more negative values as solution gate voltage increases.
We inspect this behavior for larger voltage changes as shown in Figure 3.21. As expected by the two gate asymmetrical MOSFET model (Hyung-Kyu and Fossum, 1983) there is a strong coupling between the electrodes for low accumulation regions. As the accumulation region at both interfaces grow and eventually touch, mutual threshold voltage dependence i.e. coupling becomes less prominent.

Figure 3.21 Dependence of threshold voltages of solution gate a) and backgate b) as function of the potential at the other electrode for large operating region.

Figure 3.22 shows solution gate transconductance ($g_m^{sol} = \frac{\partial i_{ds}}{\partial v_{sg}}$) for different values of backgate voltage. As expected, the peak of transconductance has shifted towards left at higher pH due to the shift of threshold voltage caused by higher pH value. The total shift is approximately 120mV for change of 4 pH units. Using this information one can estimate the sensitivity of a bioFET surface.
Figure 3.22 Double gating of a bioFET in an electrolyte solution. The 2D plots represent device transconductance as a function of gates potential at two different pH levels, the transconductance is taken with respect to solution gate voltage i.e. \( g_m = \frac{\partial i}{\partial v_{sg}} \) while keeping \( v_{bg} \) constant.

Recall that the sensitivity of a bioFET on pH changes is given by:

\[
S = \alpha \cdot 60 \frac{mV}{pH}, \alpha \leq 1
\]

where \( \alpha \) reflects the quality of the sensing surface. For Series A bioFETs, as it can be seen from Figure 3.22, the total shift of device peak transconductance due to pH change from 5 to 9 is \( \Delta V = 90 mV \). This corresponds to a change of \( \frac{\Delta \psi}{\Delta pH} \approx \frac{90 mV}{\Delta pH} = 21 \frac{mV}{pH} \). This value corresponds to the quality factor of \( \alpha = 0.37 \).
Figure 3.23 shows device sensitivity – logarithm of the current change per unit pH. It is important to notice that using both solution gate and backgate one can maximize the sensitivity of a bioFET.

Furthermore, based on Figure 3.23 one can extract solution gate threshold voltage dependence based on the choice of backgate voltage. This value is directly related to the pH sensitivity of the bioFET. As it can be seen from the figure device sensitivity drops from the maximal 21 mV/pH at the peak of transconductance ($V_{BG}=-5V$) to 17.5 mV/pH at $V_{BG}=-4V$.

![Figure 3.23 a) Device current sensitivity as a function of solution and backgate voltages. b) Device surface sensitivity (mV/pH) as a function of bias.](image)

These techniques demonstrate the importance of solution/reference gate for the bioFET sensing applications since it allows direct measurement of the device sensitivity factor. More importantly, device sensitivity can be further be tuned using the combination of solution and backgate which can potentially maximize the detection limit.

### 3.7 Summary

Demonstrated are two fabrication processes for nanowire and nanoribbon bioFETs. So far, two generations of sensors have been fabricated. The process flow was adapted to decrease device-to-
device variation in electrical characteristics and to improve the overall quality, reliability and
robustness of sensors. Several techniques for bioFET characterization and tuning are
demonstrated. The characterization shows that high quality devices with low variation in
electrical parameters can be made in a university class 1000 cleanroom. Moreover, automated
measurement systems and processing software are developed which significantly increase speed
the screening process and bring fabricated sensors from wafer to the table-top measurement
systems. More importantly, the current processes can be easily modified to allow for technology
transfer from university research lab to a commercial foundry (SOI MOSFET and FinFET).
4. Biodetection Results

4.1 Introduction
While several qualitative studies have demonstrated the true power of label-free detection method, the lack of quantitative results diminishes the competitiveness of the bioFET technology with the existing state-of-the-art techniques such as Surface Plasmon Resonance (SPR), Real-Time Polymerase chain reaction (PCR) and Luminex xMAP bead-based multiplexing. A number of previous experiments have been performed on “bottom-up” or chemical vapor deposition (CVD) grown nanowires (Patolsky et al., 2004, Li et al., 2005), but this method suffers from large device-to-device variation in electrical parameters such as threshold voltage, mobility and transconductance (Stern et al., 2005). Given these fluctuations, individual device calibration is required for quantitative analysis, thus eliminating one of the primary advantages of a microfabrication approach (i.e. multiplexing).

In order to minimize the variations of sensor electrical properties and sensing signal, two different approaches are proposed. One of them uses device solution transconductance, \( g_{m,sol} \) to normalize the total current change, \( \Delta I \), caused by analyte binding. The other applies a normalization by device baseline current level, \( I_0 \), established prior to analyte addition. We demonstrate multiplexed sensing of cancer antigens and calibration of (SOI) nanoribbon bioFETs fabricated using techniques described in Chapter 2. The top-down method accounts for the uniformity of device electrical characteristics which gives sensing repeatability and reproducibility. This aspect enables multiplexing, which allows for simultaneous data acquisition with better statistical analysis. The nanosensor surface is modified with capture-antibodies which allow specific recognition of target biomarkers.
Instead of using end-point detection to quantify device response, we instead measure initial rate, which has been shown using surface plasmon resonance (SPR) for antigen-antibody interactions to be linearly dependent on the analyte concentration (Homola et al., 1999). Furthermore, we show that initial current rate scales linearly with both device baseline current and solution transconductance, therefore supporting the need for using device electrical parameters for sensor calibration and suppression of device-to-device variation. Using the proposed method, we demonstrate universal calibration curve for device response, for a given biomarker. In addition, presented are blind test measurements performed to demonstrate the validity of the internal calibration standard.

4.2 Functionalization procedure
Plain bioFETs with an oxide insulator contain silanol groups on the surface. In order to enable sensor specificity and allow binding of biomolecular species to the sensor surface one has to confer amine or carboxylic functional groups without altering or distorting device electrical properties (Stern et al., 2007a). Typically, functionalization is done by incubating the sensor surface with 3-aminopropyltrietoxysilane in either toluene or in gas phase in a desiccator under low pressure (Vandenberg et al., 1991). The etoxy groups of APTES react with the surface hydrogen by forming ethanol and covalently bind to the surface oxygen. In order to improve the binding it is recommended to expose the sensor surface to 10 minutes long ozone plasma treatment to increase the density of the surface silanol groups. Following the 4-hour incubation period device are baked at temperatures higher than 100°C in an oven (Nagare and Mukherji, 2009).

Following the amine functionalization device are ready for receptor attachment. In our studies we focus on cancer biomarkers, a rapidly growing diagnostic tool for cancer screening(Pepe et
al., 2001). Our system was tested using Prostate Specific Antigen (PSA) and breast cancer antigen (CA 15.3). To attach appropriate antibodies to the surface we apply two equally successful functionalization schemes. One scheme utilizes glutaraldehyde as a homobifunctional linker which binds directly to the amine group thus keeping the surface functional but with carbonyl groups, (Selo et al., 1996). Upon addition of antibodies dissolved in 1× Phosphate Buffer Saline (PBS) at pH 7.4, the antibodies will bind to the sensor surface (carbonyl group) via their N-terminus. To prevent any nonspecific binding due to free carbonyl groups devices were immersed in 10mM ethanolamine solution in PBS for 30 minutes. By binding to free carbonyl groups, ethanolamine passivates the sensor surface, thus preventing any non-specific binding of proteins.

On the other hand utilizing N-hydroxysulfosuccinimide and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (NHS/EDC) chemistry one can bind the antibodies via C-terminus as well as the side-chain carboxylic groups, (Weiping et al., 1999). The difference in these two functionalization methods will be considered in detail in Chapter 5.

Upon antibody functionalization using devices were cleaned in PBS and exposed to 10% Fetal Bovine Serum (FBS) in PBS to prevent nonspecific binding.

4.3 Prefunctionalization Characterization
Prior to functionalization all chips were packaged in a 28-pin ceramic holders and wirebonded. A custom-made mixing chamber made from PTFE© tubing was mounted on each device and sealed using epoxy.

Electrical measurements, $I_{DS}$-$V_{GS}$ characteristics, were recorded using a custom-made multiplexing system prior and following the antibody attachment in order to investigate any
deterioration in sensor electrical characteristics. Figure 4.1 shows typical current and transconductance dependence on gate voltage.

![Figure 4.1 Electrical characteristics, $I_{DS}$-$V_{GS}$, and $g_m$-$V_{GS}$, of a typical nanoribbon device](image)

**4.4 Postfunctionalization characterization**

To investigate any negative effects of antibody functionalization to device characteristics, a screening was done following the functionalization.

Figure 4.2 demonstrates a typical shift in threshold voltage observed before and after immobilization of antibodies on a device surface. As expected, a shift in threshold voltage is observed due to bound charges. The measured change is on the order of 400mV measured from the backgate. This is in agreement with previous observations which measured 20-30mV (Starodub et al., 2000) from the solution gate, since the voltage changes are related through

$$\frac{\delta v_{bg}}{\delta v_{sg}} = \frac{C_{bg}}{C_{cg,dl}} \approx 0.05 \text{ (Knopfmacher et al., 2010).}$$

It is important to point out that the standard deviation of the threshold voltages of devices on a same chip stays unaltered. This tight control of the spread in $V_T$ is very important for device
calibration and analyte quantification. The standard deviation is around 30mV which is around 1%.

Figure 4.2 a) Pre- and post-functionalization I-V characteristics of a representative device. b) Distribution of device threshold voltages on a same die pre- and post-functionalization.

It is important to notice that the described method of functionalization assumes that antibodies are well and homogeneously attached everywhere. In addition, this approach should work independent of the quality of the receptor functionalization. It only requires that the functionalization – good or bad – is uniform across devices – which for a 5 by 5 mm die and 3mm mixing reservoir, usually is the case.

4.5 Biodetection
In this study devices from Series A fabrication were utilized. For all single device sensing measurements the Agilent 4156 Semiconductor parameter analyzer (SPA) was used in sampling mode, measuring $I_{DS}$ at 0.5-second intervals, and mixing was performed by manual pipetting. Devices required 1-5 minutes for current stabilization in sensing buffer, Figure 4.3.
The system we explore in our sensing experiments is the antigen-antibody interaction. Two cancer biomarker antigens of great interest for clinical research were selected – prostate specific (PSA) and breast cancer (CA15.3) antigen. Appropriate antibodies i.e. anti-PSA (Accurate Chemical, Inc.) and anti-CA15.3 (Alpha Diagnostics, Inc.) were attached to the sensor surface using the previously described procedure.

Both antigens were obtained from spiked whole blood using a microfluidic purification process (MPC) (Stern et al., 2010). Using honeycomb micropillar structure cancer biomarkers were captured from physiological solution (whole blood) and, following the washing and buffer substitution released using a UV photocleavable linker. Using a low ionic strength buffer rather than whole blood the unwanted effects such as bio-fouling, non-specific binding and degradation of sensor are avoided.

Prior to analyte addition, the mixing chamber was filled with 5 μL of pure sensing buffer. After device current stabilization, the solution to be sensed was injected. For consistency, solution addition is defined as occurring at $time = 0$. 

Figure 4.3 Typical stabilization of bioFET current as a function of time. Device is from Series A fabrication process.
Figure 4.4 shows negative control experiments i.e. the response of an anti-PSA functionalized device to a 2.25 U/mL concentration of CA15.3 in sensing buffer and vice versa, that of an anti-CA15.3 functionalized device to a 0.4 ng/mL concentration of PSA.

Figure 4.4 Negative control experiments – response of an anti-PSA functionalized device upon addition of CA15.3 (a), and vice versa, response of an anti-CA15.3 functionalized device upon addition of PSA (b).

Figure 4.5 a) and b) demonstrates normalized sensor response upon addition of 5 μL of samples containing 2.5 mg/mL PSA and 30U/mL CA15.3, and 2.0mg/mL PSA and 15 U/mL CA 15.3, respectively. The concentration of antigens in the sample was determined using an HRP-ELISA (Enzyme Linked ImmunoSorbent Assay with horse radish peroxidase as linked enzyme)). Baseline current normalization has been utilized as a traditional method in sensing.

Assuming Langmuir binding kinetics (Chapter 2) sensor response is linear in the seconds following the sample addition. If the binding is relatively strong (dissociation constant lower than pM) then it is possible to observe linear sensor response. More importantly, this initial rate is proportional to the analyte concentration at the sensor surface. If the observed system is not
diffusion limited i.e. the binding is not faster than the diffusion then what sensor sees is the bulk concentration of the analyte.

Therefore by measuring the initial current of the sensor response one can quantify the amount of analyte in the solution. Moreover, the ratios of current rates corresponding to different analyte concentration should be equal to the ratios of concentrations. In the case of PSA this value is 1.38, which is slightly above the corresponding concentrations ratios of 1.25. Similarly for CA15.3 the normalized slope rate ratio is found to be 1.94 while the ratio of concentration is 2. This demonstrates the basic idea behind the concept of signal normalization proposed in Chapter 2 which could solve the issue of device-to-device variation and internal calibration, and therefore the problem of quantification of analytes using bioFET technology.

![Graph](image)

Figure 4.5 (a)Normalized response of two anti-PSA and (b), two anti-CA15.3 functionalized devices to MPC-purified blood containing both PSA and CA 15.3, with concentrations as noted. A least squares fit is represented by a solid black line, over the selected region (line endpoints). The ratio of the normalized slopes calibrates the ratio of concentrations.

### 4.6 Multiplexed Sensing and Calibration

To further investigate the proposed method for initial current rate normalization we apply multiplexed sensing system in order to simultaneously acquire large number of data sets and investigate repeatability and reproducibility.
In a similar manner as in single device sensing measurements, prior to analyte addition (CA15.3 or PSA), 5µl of sensing buffer was left in the mixing chamber and a baseline current $I_0$ was measured. This was followed by the addition of 5µl of analyte solution upon which the current $I_{ds}$ was recorded as a function of time. The current rate was calculated numerically from recorded data after data acquisition.

Figure 4.6 shows normalized simultaneous responses of 5 devices (Series A fabrication) on the same chip upon addition of 5µl solution of 9.5U/ml CA15.3. Device signals were normalized using their baseline currents established prior to analyte injection. The operating point was $V_{BG} = -4V$, $V_{DS} = 0.1V$ and $V_{SOL} = 0V$.

As it can be seen further from Figure 4.7 device initial rates are directly proportional to the device baseline currents which are directly dependent on device electronic properties. This result confirms the validity of the proposed method. Moreover similar dependence was observed for current rates versus device transconductance.

![Figure 4.6 Normalized current response of 5 functionalized devices on the same chip upon addition of CA15.3, measured simultaneously. $I_0$ is the baseline current for a given device prior to analyte addition. The arrow indicates injection of the analyte solution. Time traces are intentionally offset by 0.05 along the y-axis for visual clarity.](image)
In order to explain the equivalence of baseline and transconductance normalization one has to go back to the fundamental FET equations for electrical current and transconductance:

\[ i_{ds} = \begin{cases} k(v_g - v_T)v_{ds}, & \text{linear region} \\ \frac{k}{2}(v_g - v_T)^2, & \text{saturation region} \end{cases} \]

and

\[ g_m = \frac{\partial i_d}{\partial v_g} = \begin{cases} kv_{ds}, & \text{lin. reg.} \\ k(v_{gs} - v_T), & \text{sat. reg.} \end{cases} \]

respectively, where \( v_g \) is the reference gate potential and \( v_T \) is the device pre-sensing threshold voltage.

Figure 4.7 Initial current rate of five devices shown in Figure 4.6 as a function of both baseline currents and device solution transconductances at \( V_{\text{backgate}}=-3\,\text{V} \) and \( V_{\text{sol}}=0\,\text{V} \). Error bars are approximately the size of the data points. The relative standard deviations for initial current rates, baseline currents and tranconductances are 0.7%, 0.3% and 0.6%, respectively. Both fits are linear \((y=kx)\), shown on a log-log scale for clarity.

The baseline current \( i_{ds0} \) and transconductance \( g_{m0} \) prior to analyte addition are linearly related by
\[ i_{ds0} = \alpha(v_g - v_T)g_{m0}, \]

where \( \alpha = \begin{cases} 1, \text{lin. reg.} \\ 1/2, \text{sat. reg.} \end{cases} \), which implies that normalizations by device baseline current \( i_{ds0} \) and by transconductance \( g_{m0} \) would yield the same results if the term \( v_g - v_T \) does not change significantly between devices. In prior work (Ishikawa et al., 2009), it was found that the scaling was better for transconductance normalization versus baseline current normalization. This is because the initial current normalization is given by \( \frac{\Delta I}{I_{ds}} = \frac{\Delta v_T}{v_{gs} - v_T} \), where \( \Delta I \) is the current change caused by the equivalent gating potential \( \Delta v \) of the absorbed biomolecules, and \( v_T \) is the threshold voltage of the device. Similarly, the transconductance scaling is given by \( \frac{\Delta I}{dV_{gs}} \). In the approach presented here the variation of \( v_T \) is insignificant (14mV SEM with \( |v_T| = 2.3V \), or < 1%), and thus initial current rate or transconductance scaling gives equivalent results.

In the approach presented here the variation of \( v_T \) is insignificant (14mV SEM with \( |v_T| = 2.3V \), or < 1%), and thus initial current rate or transconductance scaling gives equivalent results.

Figure 4.8 Calibration curves for (a) PSA and (b) CA15.3 show linear device response in the clinically relevant range of analytes. Red data point represents a blind measurement.

Measurements were repeated multiple times on different dies using different dilutions of the same 9.5U/ml stock solution. A similar procedure was repeated for PSA detection using the
serial dilutions of 10ng/ml. Applying the described normalizing method for the three analyte concentrations one can plot calibrations curves, as shown in Figure 4.8 a) and b) for PSA and CA15.3 respectively. The device response is linear in the clinically relevant concentration range (1-10ng/ml for PSA (Wu et al., 2001), 1-10U/ml for CA15.3 (Vizcarra et al., 1996)). Even though the absolute errors of the stock solution concentrations, as determined by ELISA, were relatively large (15-20% for PSA and 40% for CA15.3), the variation of device responses was less than 10% (in terms of the standard error of the mean, SEM) because the relative concentration are precise (i.e. due to serial dilutions). Each data point represents an average over multiple (7 to 10) devices. Both fits have correlation coefficients R>0.98. Table 1 summarizes the calibration results obtained for PSA and CA15.3 and demonstrates the relative standard error of the mean to be less than 10%.

In addition, we tested the calibration method using a blind measurement on a single device and a different measurement setup (red circle, Figure 4.8) and compared it to the calibrated data. The blind measurement was obtained from the stock solution. Upon introducing a concentration of 0.4±0.08 ng/ml of PSA, as determined by conventional ELISA, we measured a normalized current rate of $1.0054 \times 10^{-4}$ s$^{-1}$. Using the calibration curve shown in Figure 4.8 we calculated the expected value to be 0.46±0.06ng/ml. This measurement deviates from the concentration obtained by using conventional ELISA by approximately 15%.
<table>
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<tr>
<th>PSA concentration [ng/ml]</th>
<th>Number of devices</th>
<th>Normalized device signal, average [s⁻¹]</th>
<th>Standard error of the mean [s⁻¹]</th>
<th>% error</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.3 ± 0.7</td>
<td>7</td>
<td>3.225 x 10⁴</td>
<td>2.9 x 10⁵</td>
<td>8.8</td>
</tr>
<tr>
<td>5.0 ± 1.0</td>
<td>10</td>
<td>5.179 x 10⁴</td>
<td>5.1 x 10⁵</td>
<td>9.9</td>
</tr>
<tr>
<td>0.22 ± 0.04</td>
<td>8</td>
<td>7.869 x 10³</td>
<td>7.5 x 10⁶</td>
<td>9.6</td>
</tr>
<tr>
<td>CA 15.3 concentration[U/ml]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.75±1.8</td>
<td>7</td>
<td>1.32 x 10³</td>
<td>5 x 10⁵</td>
<td>3.8</td>
</tr>
<tr>
<td>3.2±1.2</td>
<td>8</td>
<td>8.6 x 10⁴</td>
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<td>5.8</td>
</tr>
<tr>
<td>0.5±0.2</td>
<td>9</td>
<td>1.65 x 10⁴</td>
<td>1 x 10⁵</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 1 Calibration points for PSA and CA15.3 obtained on multiple devices demonstrating the relative standard error of the mean to be below 10%.

### 4.7 Size Scaling

BioFET response dependence on size has been a long standing debate discussed in both theoretical (Nair and Alam, 2007) and experimental papers (Fan et al., 2004). It has been previously shown using CVD grown nanowires in label-free detection of inorganic molecular species in gas phase that this dependence follows an inverse diameter law (Fan and Liu, 2006). However, these experiments did not have good control over nanowire electrical parameters, therefore any dependence of electrical characteristics on device size would directly affect the sensitivity.

Figure 4.9 shows parameters such as threshold voltage and subthreshold swing for Series B nanowire bioFETs fabricated using EBL. As expected there is no dependence on device width at the order of tens of nanometers (Ahmed and Vasileska, 2003). This is very important since if all devices are working under the same bias condition, any significant dependence of sensitivity on device size would be a consequence of geometrical and not electrical parameters.
To explore device pH sensitivity scaling we used devices on a same chip under same conditions – bias, reference gate potential, and buffers. Buffers (0.01X PBS) with various pH values were exchanged and the change of current $\Delta I$ was recorded as well as the solution gate threshold voltage shift before and after the exchange. Using the change in surface potential (solution gate threshold voltage shift) one can determine the pH sensitivity ($S_{pH} = d\psi / dpH$) of a bioFET. The dependence of pH sensitivity on device width is shown in Figure 4.9. The sensitivity increases when the width becomes on the order of 100nm or less. This effect can be explained by the scaling model presented in Chapter 2, and is the Debye screening length in silicon. When the device width $w$ is much larger than the screening length $\lambda_{D,Si}$, the channel is not entirely affected by the bound charge. However, when the channel width becomes on the order or smaller than the $\lambda_D$ the effect of the side-walls charge would affect the whole channel and thus the sensitivity would increase, Figure 4.10.
Similarly, we investigate device sensitivity $S = \Delta I/I$ scaling using the anti-PSA/PSA system described in Chapter 3. Upon establishing a baseline current, PSA was added and current was recorded. Figure 4.11 shows device sensitivity as a function of channel width. As previously described sensitivity increases as device width becomes on the order of Debye length in silicon.

It is also important to notice that one could expect this effect due to the definition of sensitivity as $S = \Delta G/G$. If device thickness or width is bigger than the Debye length then surface binding of charge would not affect the whole channel. When device cross-section become small enough
the whole channel is affected by the surface charge while the channel conductance is minimized therefore maximizing the ratio $\Delta G/G$.

Figure 4.11 also demonstrates the discrepancy between the model and experimental data. As previously explained in Chapter 2, this is effect expected when Debye screening length in silicon is on the order of device thickness and sensitivity as a function of thickness reaches maximum value. When the device width reaches values of $2-3 \lambda_{D,\text{Si}}$ the effect of side-walls starts contributing to additional increase in sensitivity.

4.8 Summary
Successful demonstration of a novel method for internal calibration of bioFETs is presented and its application to quantification of clinically relevant analytes i.e. cancer biomarkers PSA and CA15.3, is explored. Described approach is enabled by the ability of the CMOS-compatible fabrication method we have developed to produce devices with very similar transport characteristics. Initial kinetic rates, proportional to the initial sensor current rate, were utilized rather than the endpoint detection. Measured calibration curves show linear response in the relevant concentration range as well as good agreement with a blind measurement. In addition, we demonstrate that calibration by baseline current normalization is equivalent to that obtained by transconductance normalization in case of low variation of threshold voltages between devices. Our results demonstrate that nanosensors fabricated by conventional CMOS compatible processes yield reproducible results traceable to a calibration standard. We believe that this approach will make nanoscale FET sensor technology a step closer to commercial point-of-care applications. More importantly, we demonstrate that device sensitivity scaling is directly related to the Debye screening length in silicon and becomes more prominent as width and thickness of bioFET reach the same order of magnitude as Debye length. In the case of thickness, sensitivity
depends as $\sim 1/t$ for thicknesses that are greater than Debye length in silicon. For thicknesses around $\lambda_{D,\text{Si}}$ the sensitivity reaches maximum value. When thickness is kept constant and is on the order of $\lambda_{D,\text{Si}}$ which maximizes the sensitivity, similar $\sim 1/w$ dependence is observed for widths that are on the order of $\lambda_{D,\text{Si}}$, however for larger widths there is a discrepancy between the model and experimental data. This is expected since the side-wall charge is enhanced at widths that are on the order $\sim \lambda_{D,\text{Si}}$. 
5. Debye Length Modulation Technique

5.1 Introduction
As mentioned before, in label-free detection schemes the nanowire surface is functionalized with specific receptors capable of recognizing and capturing specific target molecule. Upon binding, charges on the capture-molecules modulate the nanowire’s surface potential. This change in the electrical field causes accumulation or depletion of carriers in the bioFET channel thus decreasing or increasing nanowire current. In ionic solution, however, dissolved charged species form an electrical double layer, lowering the effective charge of the biomolecules. This effect is known as Debye screening (Figure 5.1) and has exponential behavior \( \sim \exp \left( \frac{-x}{\lambda_D} \right) \), with a characteristic distance parameter known as the Debye screening length, \( \lambda_D \), defined by (Israelachvili, 1991):

\[
\lambda_D = \frac{1}{\sqrt{4\pi l_B \sum \rho_i z_i^2}}
\]

where \( l_B \) is the Bjerrum length (0.7 nm) and \( \rho_i \) and \( z_i \) are the density and the valence of the \( i^{th} \) ionic species. For typical biological buffered solutions (i.e. 1× to 0.1× phosphate buffered saline, PBS), \( \lambda_D \) is approximately 0.7 to 2.2 nm. Experimental studies have demonstrated that these short distances do not significantly affect the detection of small molecules such as DNA or RNA oligonucleotides (~2nm) (Bunimovitch et al., 2006, Li et al., 2004, Zhang et al., 2008). However, for larger macromolecules, such as antibodies, size suggests that FET based detection of antigens via specific binding to antibody-functionalized surfaces will be greatly affected by Debye screening (Nair and Alam, 2007). The first experimental demonstration of the effect of Debye screening on nanowire-based biomolecule detection was performed using a biotinylated...
sensor for specific detection of streptavidin ligand (Stern et al., 2007b). This work demonstrated that buffer conditions ranging from high to low salt concentrations can severely impact detection sensitivity via Debye screening.

![Debye screening effect](image)

**Figure 5.1 Basic principle behind the Debye screening effect.** An increase in buffer ionic strength decreases Debye length and the effective charge of bound molecule as seen from nanosensor surface.

In this work, we extend the understanding of the effect of Debye screening from the model biotin-avidin system to the antibody-antigen systems shown in Chapter 4. We examine the effects of ionic strength of the sensing buffer on the level of signal obtained upon label-free detection of a model biomarker, the breast cancer biomarker (CA15.3) using silicon nanoribbon bioFETs (Elfstrom et al., 2008, Stern et al., 2010). In addition, a theoretical model has been developed to explain observed measurements and to allow extraction of relevant information such as average spatial extent of charges on the molecule.

To demonstrate how antibody orientation on the sensor surface impacts sensing, two different immobilization schemes that bind at different termini are used. The resulting different antibody arrangements produce different sensor-to-antigen binding site distances, allowing us to perform a detailed study of the effect of charge screening on sensor response.
5.2 Device Surface Functionalization

Amine functionality was conferred to the device surface using an already known technique - devices were functionalized using 3-aminopropyltriethoxysilane (APTES). The APTES layer was formed by immersing the wafers in 5% (v/v) APTES in toluene for 2 hours in a nitrogen atmosphere. To improve the monolayer stability, wafers were baked at 180°C for 2 hours in a vacuum oven.

Anti-CA15.3 (Alpha Diagnostics) was immobilized on the nanoribbon surface by coupling to either the C- or N- terminus, Figure 5.2 (Weiping et al., 1999, Selo et al., 1996). Coupling to APTES-functionalized devices via the C-terminus was achieved using N-hydroxysulfosuccinimide and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (NHS/EDC) chemistry in 1X phosphate buffered saline (PBS; Sigma) at pH 7.4 (Nagare and Mukherji, 2009). Samples were then washed with 1X PBS and blocked with 10% fetal bovine serum (FBS) for 30 minutes, followed by washing with sensing buffer (1mM bicarbonate buffer at pH 9). Due to nonselectivity of the NHS/EDC chemistry, the surface configuration of the antibodies results in an ensemble of different orientations due to the coupling of the C-terminus and side chain carboxylic groups with the sensor surface.

To couple the antibody via N-terminus, APTES-functionalized devices were immersed in a 5% glutaraldehyde solution in deionized (DI) water for 2 hours at room temperature, Figure 5.2. After washing with 1× PBS, the device surface was reacted with anti-CA15.3 in 1× PBS at pH 7.4 for 2 hours, to yield a different antibody arrangement on the surface (Selo et al., 1996). A pH 7.4 ensures that the N-terminus is deprotonated (available for reaction) and the side chain amines are protonated (unavailable for reaction), whereas higher pH (i.e. pH 9.0) would allow all amines (N-terminus and lysine side chains) to be deprotonated (thus an ensemble of configurations).
Thus, this allows the bound antigen to be in closer proximity to the sensor surface and be less affected by Debye screening. Unreacted glutaraldehyde was quenched with ethanolamine and the surface was subsequently washed with PBS. The sample was then blocked with 10% FBS for 30 minutes, followed by washing with 1mM bicarbonate sensing buffer. Following washing of all samples, 10µl of sensing buffer was left in the mixing chamber.

Figure 5.2 Antibody Immobilization. Schemes of antibody immobilization on the sensor surface resulting in different antibody arrangement. Antibodies are immobilized either by the C-terminus or carboxylate containing side chains using NHS/EDC chemistry, or the N-terminus using glutaraldehyde.

5.3 Sensor response in presence of Debye Screening

Presence of charged ionic species in the buffer solution is necessary to allow proper functioning of biomolecules. However, as it was shown in Chapter 2, these ionic species cause electrostatic screening of biomolecular species thus lowering their effective charge as a function of distance. This means that the effective signal measured by the bioFET will directly depend on the ionic strength of the buffer and the distance between the receptor and the sensor surface. It is important to mention that the shape of the proteins at low concentration levels and low ionic strengths of buffers would not change significantly (Zhang et al., 2006).
The change of conductance of a bioFET due to the binding of surface charge $\sigma_S$ at distance $l$ from the surface in a buffer solution with Debye screening length $\lambda_D$ is given by:

$$\frac{\Delta G}{G_0} = \Delta I \frac{I(\lambda_D) - I_0}{I_0} = \Gamma l \sigma_S$$

Parameters $\Gamma$ and $\Gamma_l$ are sensitivity factors which describe sensor response due to charge binding and Debye screening, respectively. For nanoribbon sensor $\Gamma_l$ is given by (Sorensen et al., 2007):

$$\Gamma_l = 2 \left[ 1 + \exp \left( \frac{l}{\lambda_D} \right) \right]^{-1}$$

By measuring device response upon binding as a function of buffers with various ionic strengths one can extract the Debye sensitivity factor $\Gamma_l$ and determine average spatial extent of the bound charge from the nanosensor surface ($l$). Described procedure enables probing of the surface configuration of bound molecules and could be utilized for quantitative assessment of the bound molecules including dynamic measurements of conformational changes.

### 5.4 Debye Length modulation technique

All sensing measurements were performed using the DAQ system with $V_{ds} = 0.2$ V, $V_{\text{backgate,s}} = -3$ V, $V_{\text{solutiongate,s}} = 0$ V and a sampling rate of 0.5 sec. 10$\mu$l of antigen solution was injected after establishing a stable baseline current.

The response of an anti-CA15.3-functionalized sensor to the addition of 10 $\mu$l of 50 U/ml CA15.3 in 1mM bicarbonate buffer at pH 9 ($\lambda_D = 9.7$ nm) is given in Figure 5.3a. The binding of the negatively charged antigen (the isoelectric point, $pI$, of CA15.3 is a pH<5 (Wu et al., 2008)) causes an increase in the current of the p-type device. After the device current stabilizes, the 1 mM buffer was exchanged with a 0.1 mM bicarbonate buffer with the same pH, thereby
increasing the Debye length to 30.7 nm. This exposes more of the antigen’s charge to the sensor, further increasing device current, Figure 5.3a. The buffer was then replaced with a 1 mM bicarbonate buffer with 10 mM NaCl at pH 9 ($\lambda_D = 3\,\text{nm}$). The resulting increase in ionic screening causes a steep decline in current since the Debye length for this ionic strength is shorter than the typical antibody size. Furthermore, since Debye screening exhibits an exponential behavior, the current does not fully drop to the pre-antigen binding level. The same buffer exchanges were performed on sensors in which the anti-CA15.3 was bound through its N-terminus, Figure 5.3b.

![Figure 5.3 Debye Length Modulation](image)

**Figure 5.3 Debye Length Modulation.** Time domain device response after antigen injection in 1 mM sensing buffer, followed by a buffer exchange to 0.1 mM and 10 mM for devices functionalized with anti-CA15.3 via their a) C-terminus or b) N-terminus.

It is important to notice that the buffer exchange will not affect the amount of bound charge as long as the dissociation time of the antigen is much longer than the typical time scale for these experiments. Instead of relying on literature values for the unbinding rate, $k_{off}$, of the system used (CA15.3), we directly determined this under the exact same experimental conditions, and using the same antibody/antigen batch used in the Debye length measurements, using an SPR Biacore T100 system for a real-time measurement of the dissociation constant (Malmqvist,
By measuring typical binding/unbinding curve for CA15.3 measured in 0.1M HBS buffer one can determine the dissociation rate in different buffers. There are four characteristic regions observed on the sensogram. First a pre-binding period where the signal is stabilized, second the binding event when ligand is added to the system, third the unbinding event when the analyte (ligand) solution is substituted with pure buffer which causes shift in equilibrium and therefore the unbinding of the ligand and, lastly, the fourth region when a low pH value solution is added to the system to release the bound ligand and regenerate the surface and capture antibodies for the repetition of the experiment.

In order to determine the off-rate constant for a given antibody we use well known receptor-ligand binding kinetics. In the simplest version of this model a single ligand L binds to a single receptor R forming a complex C i.e. \( R + L \leftrightarrow C \) with forward (on rate) and backward (off rate) constants \( k_{on} \) and \( k_{off} \), respectively. In purely unbinding events (no presence of ligand when the system is flushed with buffer) one can observe exponentially decaying dependence of SPR signal over time i.e. \( \sim \exp(-k_{off}t) \). This defines the unbinding (off) rate of the ligand and can be obtained by linearization.

In 0.1M buffer, the dissociation (off-) rate of CA15.3 is measured to be \( k_{off} = 3.71 \cdot 10^{-7} \text{s}^{-1} \) which is equivalent to a time constant of \( \tau=2.7 \text{Ms} \). Similarly we measured the off rate in 10mM and 1mM bicarbonate buffers and found them to be \( k_{off} = 2.43 \cdot 10^{-6} \text{s}^{-1} \) and \( k_{off} = 1.17 \cdot 10^{-6} \text{s}^{-1} \). Even though changing the concentration of the buffer modifies values of dissociation rate, the effect upon our result is negligible. The time constants corresponding to different buffer concentrations range from \( \sim850\text{ks} \) to \( \sim2.7\text{Ms} \), whereas the typical time scale of our experiments is 500-1000s, thus, the maximum relative change of device signal due to this effect is \( \sim0.1\% \).
Using the proposed Debye screening model and a nonlinear least squares method, we estimate that $\Delta I_{\text{max}} = 7.3 \text{nA}$, Figure 5.4a. Similarly, using the data in Figure 5.4b, we estimate that $\Delta I_{\text{max}} = 1.52 \text{nA}$ for N-terminal antibody functionalization.

Figure 5.4 Nonlinear fit of device signal change versus the Debye length according to equation (3) in the main text. Fitting parameters are $\Delta I_{\text{max}} = 7.3 \text{nA}$ and $l = 8.0 \text{nm}$ for a) and $\Delta I_{\text{max}} = 1.52 \text{nA}$ and $l = 6.3 \text{nm}$ for b).

Ideally when the surface charge density is located at the nanosensor surface, one can estimate the upper boundary for $\Delta I_{\text{max}}$ for a specific device. We use the approximation that $\Delta I_{\text{max}} = (\partial I / \partial \psi_0) \Delta \psi_0$, where $\psi_0$ is sensor/solution interface potential and the derivative represents the solution-gate transconductance of the device. The relationship between the surface charge density, $\sigma_0$, of the bound molecules on the sensor surface and the potential at the sensor/solution interface, $\psi_0$ (Israelachvili, 1991) with respect to the reference electrode is:

$$\sigma_0 = \sqrt{8 \epsilon_0 \epsilon_W k_B T c_0} \sinh \left( \frac{e \psi_0}{2k_B T} \right)$$

where $\epsilon_0$ is the vacuum permittivity, $\epsilon_W$ is the relative permittivity of water, $k_B$ is the Boltzmann constant, $T$ is the temperature and $c_0$ is the density of ionic species in the solution. The change in
the nanosensor’s surface potential, $\Delta \psi_0$, is caused by antigen binding and change of surface charge density. The change of surface charge density is equal to the charge of bound antigens per surface area.

This value is estimated to be approximately $-10e$ using the Scripps institute Protein Calculator v3.3 at pH 9 and the UniProt peptide sequence library (see Appendix II). This leads to an average surface sheet charge of $\Delta \sigma_0 \approx 2e/nm^2$, where $e$ is the unit charge. Using $\varepsilon_W = 80$, $c_0 = 6 \times 10^{23} m^{-3}$, and $k_B T / e = 26 mV$, we estimate $\Delta \psi_0 = 30 mV$. The solution gate transconductance of the device shown in Figure 5.4b is approximately 80 nA/V, as measured directly by solution gating. This yields $\Delta I_{max} \approx 2.4$ nA and is in good agreement with the value obtained from experimental data $\Delta I_{max} = 1.52$nA.

Using this information we calculated the percentage of unscreened signal i.e. $\Gamma = \frac{\Delta I}{\Delta I_{max}}$ for two functionalization schemes using set of 5 different devices for each schemes, Figure5.5.

![Figure 5.5 Percentage of unscreened signal for N- and C-terminus surface functionalization for 10 total devices (5 each).](image)
Using the data from multiple devices and the linearized equation \( \frac{\Delta I}{\Delta I_{\text{max}}} = 2\left[1 + \exp\left(\frac{l}{\lambda_D}\right)\right]^{-1} \), we obtained values for the bound charge average distance from the nanosensor surface, \( l = (8.4\pm0.4) \) nm and \( l = (5.9\pm0.6) \) nm for the for C- and N-termini-bound antibodies, respectively. These results are summarized in Figure 5.6. \( I_l \) is calculated using the \( \Delta I/\Delta I_{\text{max}} \). The result obtained for C-terminus functionalized devices are in agreement with the value obtained using atomic force microscopy (Park et al., 2009). The error bars are calculated in terms of the standard error of the mean. The estimated lengths are in excellent agreement with typical antibody-antigen complex dimensions, specifically their height and diameter, respectively. Therefore, this approach enables a measurement of the average distance (with respect to the sensor surface) of charged species in receptor-ligand complexes.

![Figure 5.6 Linearized dependence of the sensitivity factor \( I_l \) as a function of Debye length of the sensing buffer for different schemes of functionalization compared to theory.](image)

By definition \( I_l \) describes the percentage of the surface charge seen by the device in the presence of Debye screening therefore it describes the percentage of the unscreened signal at given Debye
length, $\Delta I/\Delta I_{\text{max}}$. We estimate that $\sim 50 \pm 3$ % (5 total devices) of antigen charge is exposed to the sensor (i.e. unscreened) when using 1mM bicarbonate sensing buffer for C-termini-functionalized antibodies. The shown error is calculated in terms of the standard error of the mean (SEM). Through using N-terminal functionalization, the percentage of exposed charge increased to $\sim 65 \pm 2$ % (5 total devices). This demonstrates the influence of binding site distance from the sensor surface on signal detection, resulting from differences in antibody configuration.

5.5 Summary
Debye screening manipulation can be employed for quantitative spatial analysis of induced charge on a nanosensor surface. The explored model system was a functionalized receptor (antibody) - target ligand (antigen), but the approach detailed here holds for any bound charged moiety. Specifically, we show that different configurations of receptors can be distinguished by the Debye screening manipulation method. In addition to the enhanced quantitative understanding of analyte-receptor spatial configuration, this approach also opens new directions for FET based nanosensor applications, such as observing dynamic conformational changes in biomolecules. Presented results further demonstrate the critical dependence of sensitivity on receptor orientation, highlighting the importance of functionalization chemistries.
6. Conclusion

To date, numerous papers on silicon field effect nanosensors have been published in scientific journals and conferences. Most of these articles deal with qualitative sensing where FET sensing platform is applied over and over again to different biological systems varying from protein and oligonucleotide to detection of single viruses and cellular functions. Even though the whole spectrum of applications has successfully been covered, three fundamental topics were never assessed:

1) can sensors be calibrated; and if so,

2) can they be used for quantitative, repeatable and reproducible measurement of analytes; and if so

3) how this quantitative response depends on the devices geometrical and electrical parameters.

This work tries to give reasonable answer to all three issues.

First and foremost, we demonstrated successful fabrication of silicon bioFET arrays capable of multiplexed simultaneous detection of analytes. Using CMOS compatible processes we achieved very precise control over sensor electrical properties such as threshold voltage. The variation achieved on the wafer scale was on the order of 5% which, for the Class 100/1000 cleanroom where the fabrication was performed, is an extreme success. Moreover, the yield of the devices following the fabrication process was on the order of 90%. Having good quality devices is a prerequisite for investigation of reproducibility and repeatability of sensing experiments.
Second, we developed a theoretical model for nanowire bioFET-electrolyte model that successfully managed to explain effects of Debye screening and surface charge on nanosensors source-drain current. In addition we related the magnitude of current change to device electrical parameters such as transconductance. By applying this model and the ligand-receptor binding kinetics we demonstrate that nanosensor current rates in the seconds/minutes upon analyte addition are directly proportional to analyte concentrations. This establishes a new method for analyte quantification in addition to the end-point detection. More importantly, the application of the new method on cancer biomarker detection such as prostate specific antigen (PSA) and breast cancer antigen (CA15.3) yields tremendous results. Relative standard deviation of the measurements is below 10% on the samples obtained by serial dilution of the stock solution. Result obtained by traditional optical ELISA had relative standard deviation of 25% for PSA and up to 40% for CA15.3 at clinically relevant concentration range. This result, first of its kind in bioFET technology, demonstrates the true power of the electronic label-free detection, and paves the way for future clinical and commercial applications.

Using the Debye screening model for bioFET we related device response as a function of bound charge distance and ion strength of the sensing buffer. This approach evolved in Debye length modulation (DLM) technique which allows one to probe the charges on a sensor surface and therefore explore their effective spatial extent. In addition, we showed that using DLM one can distinguish between different surface configurations of bound molecules which arise from utilization of different functionalization techniques. This opens up a new direction in field effect biosensor applications such as direct measurement of conformational changes in molecules due to denaturing or cooperative binding.
Lastly, this work goes a step further by trying to explain scaling of response with sensor size. By utilizing the Poisson-Boltzmann model for semiconductor-oxide-electrolyte system we conclude that critical role in transduction of the surface charge to the nanosensor is played by the Debye screening in the semiconductor itself. By having less doped (i.e. less metallic) semiconductor material more of the surface charge is reflected in the semiconductor and therefore the effect of conductance modulation is greater. Critical role is played by the definition of sensitivity as $S = \Delta G/G_0$. By decreasing geometrical dimensions of nanosensor we decrease the baseline conductivity $G_0$ and increase the total change $\Delta G$ since relatively greater part of the device channel is affected by the surface charge. At dimensions that are on the order of the Debye screening length, sensitivity reaches maximum value. More importantly, we confirm our model by experimental measurements of both pH and label-free sensitivity of bioFET.

The result presented in this thesis put semiconductor bioFET technology a step closer to the already existing cutting-edge techniques such as surface plasmon resonance. By demonstrating device calibration and quantitative sensing as well repeatability and reproducibility, we believe that this technology will have potential applications in the future as point-of-care diagnostic tool.

However, there are still several points to be addressed by researchers.

1) Fundamental: the definition of sensitivity as $S = \Delta G/G$ gives an unfair advantage to scaling and, as we demonstrated before it yields that smaller devices are more sensitive compared to their larger counterparts. However, the final answer to this question can be given by exploring noise characteristics and signal-to-noise ratio of nanoscale versus microscale devices, which could be built on the theory developed here. In addition, an investigation of the noise in bioFET
will ultimately give an answer whether this technology would be able to ever detect single molecules or cells.

2) Technical: even though we demonstrated successful integration of nanosenors into arrays and multiplexed sensing of biomarkers, there is still not an efficient way of integration with microfluidics which would allow high throughput sensing and diagnostics.

Both of these challenges are currently being addressed, and results so far have been very promising. It is very possible that in the near future we would be able to use these devices in our day-to-day life integrated with laptops and even smartphones.
Appendix

A1. Nanelectronic ELISA

Device Fabrication
Indium oxide nanowires were chosen as sensor material due their previous demonstration as biosensors (Li et al., 2005). These nanowires were grown using laser-ablated hot-wall chemical vapor deposition method using a gold catalyst (Liu et al., 2003). Devices were fabricated on degenerately doped 2” silicon wafers previously thermally oxidized to achieve SiO₂ thickness of 200nm. The nanowires, grown on Si/SiO2 substrate, were suspended in semiconductor-processing-grade isopropanol (Brand Nu Labs) by ultrasonic agitation. In the first lithography step, a lift-off bilayer of photoresists LOR5A/Shipley1808 is spun followed by an exposure, development and 5:1 Buffered Oxide Etch (BOE, T.J. Baker) to form a backgate via. Next, a Ni/Au metal layer is evaporated using an electron-beam evaporator (Denton Vacuum System) followed by a lift-off process (N-methyl pyrrolidone, VWR Scientific) and rapid thermal annealing at 400°C for 2 minutes in N₂ atmosphere to improve Ohmic behavior of contacts. The nanowires were then deposited from isopropanol solution and metal (Ni/Au) leads were patterned using the same lift-off process used for backgate definition, Figure A.1. Prior to metal deposition nanowire surface was cleaned from photoresist residue by ozone plasma for 1 minute at 100W of power at 0.350 Torr of oxygen pressure. To lower the contact resistance, nanowires were annealed at 400°C for 1 minute in nitrogen atmosphere. After preliminary screening of the electrical characteristics, the wafer was diced into 3x3mm dies and packaged on a 16-pin DIP header and wirebonded.
A polydimethylsiloxane (PDMS) mixing cup was mounted on each chip and surface functionalization was performed. In order to fabricate mixing chambers we used the silicone elastomer base and curing agent from the PDMS kit (Dow Corning) and mixed them in a weigh boat in a 10:1 (w/w) ratio. The boat was then placed in a dessicator to remove air bubbles (~10 min). The solution was poured onto a microscope slide to a level of ~5 mm above the glass slide and was subsequently baked for two hours at 65°C to harden the PDMS (Duffy et al., 1998). A metal tube with a 1.5 mm outer diameter (Small Parts, Inc.) and a sharpened end was used to punch holes in the PDMS and a razor blade was used to free the gaskets from the PDMS sheets. Theses gaskets were pressed onto dies to create the sensor wells (Figure A.2).
Device Functionalization
Samples were then treated with ω-mercaptocarboxylic acid to confer carboxylic functionality to the gold leads through thiol-mediated self-assembled monolayer (SAM) formation as shown previously (Bain and Whitesides, 1989), Figure A.3. In this work, gold leads contacting the nanowires were used for convenience; however, any exposed gold surface in proximity to the sensor surface could be utilized. In the same time, the gold surface is passivated by the SAM formation, which can be demonstrated using cyclic voltammetry (Chidsey and Loiacono, 1990). In Figure A.4 the redox peaks due to the exposed gold surface are significantly reduced after functionalization, indicating the formation of a functional surface. The gold coated substrates used in cyclic voltammetry (CV) experiments were prepared by an E-beam evaporation of a 5 nm Cr (99.9%, Kurt J. Lesker Co.) / 70 nm Au (99.99% Cerac, Inc.) stack onto Si wafers (Silicon Quest International) previously cleaned with
Figure A.3 Surface functionalization schematic. The steps are described in the text.
piranha [1:3 H₂O₂ : H₂SO₄ (J.T. Baker Co.)], acetone (Sigma), methanol (Sigma), and deionized water (DI) and then blown dry with nitrogen.

The substrates were then immersed in a 1 mM solution of ω-mercaptocarboxylic acid prepared in deoxygenated, absolute ethanol and left for 12hrs in the dark in an inert atmosphere. Samples were subsequently washed with ethanol, toluene (Sigma), and isopropanol (Sigma) and blown dry with a directed stream of nitrogen. The CV measurements were performed using a Gamry Femtostat using a Pt counter electrode (Ernest F. Fullham, Inc.) and an Ag/AgCl reference electrode fabricated by the electrodeposition of AgCl on an Ag wire (Ernest F. Fullham, Inc.) from a saturated aqueous solution of NaCl (Sigma).

SAM formation on the gold leads minimally affects the electrical properties of the FETs. This is shown in the pre- and postfunctionalization dependence of the drain-source current on drain-source voltage, I DS(V DS), for varying gate-drain voltage, V GD, in Figure A.5. The insets show the I DS(V GS) dependence for the V DS=0.5V operating point used in all sensing experiments. The postfunctionalization characteristics show that SAM formation does decrease the threshold voltage (V t, thus increasing I DS for set V GD in postfunctionalization device), but has a minimal effect on the transconductance at the operating point used for sensing (V GD=0). Additionally, the subthreshold leakage current does slightly increase with V GD but to a level 100-fold lower that used for sensing.
Functionalized devices were first treated with a 0.1 mg/mL solution of the capture anti-IL-2 antibody (BD Biosciences; Cat. No. 555051) in PBS with 0.05 mg EDC and 0.025 mg N-hydroxysulfosuccinimide (sulfo-NHS; Pierce Scientific) for 1 hr. Washing was performed three times using PBS, followed by the addition of a 10% bovine serum albumin (BSA; Sigma) solution, after which washing was again performed. Next, IL-2 (Novartis) was added at varying concentrations as described in the text for 1 hr. Periodic pipetting up-and-down was performed to maximize protein binding. After washing three times with PBS, a 0.1 mg/mL solution of avidin-conjugated anti-IL-2 detection antibody was added for 1 hr. This conjugate was generated by adding equal molar amounts of streptavidin (Pierce Scientific) and biotinylated-anti-IL-2 (BD Biosciences, Cat. No. 555040) and allowing the binding to occur for 15 minutes at room temperature in PBS. The conjugates were stored at 4°C and used without further purification. After washing three times with PBS, a 0.1 mg/mL solution of biotin-urease in PBS was added for 1 hr. Washing was again performed three times with PBS and devices were then washed and
stored in the sensing buffer (0.01X PBS + 150 mMNaCl, pH 8.0) directly prior to use. The sensing buffer was titrated to pH 8.0 with 1 N solutions of HCl (J.T. Baker) and NaOH (J.T. Baker).

Figure A.5 (a) Unfunctionalized and (b) functionalized ω-mercaptopcarboxylic acid \( I_{DS}(V_{DS}) \) characteristics for \( V_{GD} \) increased from –25V to 25V in 5V increments for a single representative device. The arrow shows increasing \( V_{GD} \). The insets show the \( I_{DS}(V_{GD}) \) characteristics for \( V_{DS} = 0.5V \) for the same device and the arrows indicate the sweep direction. The operating point for sensing measurements was \( V_{DS} = 0.5V \) and \( V_{GD} = 0V \).

We have seen that the calibration method we proposed works in favor of reducing device-to-device variations and allows analyte quantification based on the normalized current rate. To further, demonstrate the true power of the method we device a indirect assay (unlike more commonly used label-free one) and apply it to CVD-grown nanowires which, as previously mentioned, posses more variation in its electrical parameters. Since the new method uses a sandwich assay similar to the Enzyme-Linked ImmunoSorbent Assay in biochemistry we term it a nanoelectronic-ELISA or ne-ELISA.

The principle of the ne-ELISA is illustrated schematically in Figure A.6. Urease (here, bound via neutravidin-biotin to the secondary antibody) hydrolyzes free urea in the nanosensor reservoir according to the following reaction:
Thus, introduction of urea to the reservoir raises the solution pH, thereby decreasing $I_{DS}$. Urea is added at a sufficient concentration for the urease to catalyze Eq. A.1 ($\sim 10^5 : 1$ molar ratio of urea to urease) at its maximum velocity, thus the rate of change in $I_{DS}$ will correlate directly with the quantity of bound urease and, in turn, bound IL-2.

Figure A.6 Schematic of the ne-ELISA approach. Before the addition of urea the indium oxide surface is relatively protonated, inducing a relatively large channel current (large white arrow). The addition of urea results in the removal of protons from the solution and, thus, increased deprotonation of the nanowire surface. This, in turn, induces a decrease in channel current (small white arrow).

Figure A.7 $I_{DS}$ vs. $V_{GD}$ dependencies for a solution-gated (red) device. Solution gating was performed in the pH 8.0 buffer described in the text. The green dataplot shows the leakage current of the device ($I_{LEAKAGE}$) during the solution-gating measurement.
To demonstrate the capability of the ne-ELISA for detection of labile macromolecules, we focused on the cytokine interleukin-2 (IL-2), whose presence reports on the activity of the T cell immune response. In the initial step, a capture IL-2 monoclonal antibody was conjugated to the carboxylic groups on the gold leads through its N-terminus (Figure A.3-ii). This was followed by a wash step that removed unbound capture antibodies (all binding steps described below are followed by washes) and the subsequent placement of a PDMS gasket over the nanowire devices to create the sensing reservoir. Next, a bovine serum albumin solution was used to prevent nonspecific protein adsorption to the chip and reservoir sidewalls, which is a typical blocking step used in conventional colorimetric ELISA protocols to minimize non-specific binding. This was followed by the addition of IL-2 at varying concentrations (across different devices) to the reservoir (Figure A.3-iii). A secondary, biotinylated antibody to IL-2 was then introduced (Figure A.3-iv), followed by the addition of neutravidin, a tetravalent biotin-binding protein, and biotinylated urease (Figure A.3-v).

The biotin-urease conjugate was made in-house using standard process: (+)-biotinamidohexanoic acid hydrazide (biotin-LC-hydrazide; 3 mg; Pierce Scientific) was dissolved in 500 μL DMSO (Sigma) and added to a solution of jack bean urease (1 mg/mL; Sigma, Cat. No. 94285) and 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC; 5 mg/mL; Pierce Scientific) in 3 mL phosphate buffered saline (PBS; Sigma). The reaction proceeded for 1 hr at room temperature with shaking. After the conjugation was complete, the product was isolated by centrifugation using a 50 mL Amicon Ultra (Millipore, Cat. No.UFC905024) centrifugal filter tube with a 50,000 MW cutoff (four 30-minute spins at 3000 rpm at 4°C). The resulting biotin-urease was diluted to 0.1 mg/mL and stored at 4°C.
Application of the Calibration Technique to bottom-up Nanowire bioFETs

We first characterized the pH sensitivity of the In$_2$O$_3$ nanowires. Conventional indium tin oxide ISFETs have been previously demonstrated to have a linear pH sensitivity between pH 2 and 12, thus we expected undoped In$_2$O$_3$ to exhibit a similar response. The response of a characteristic device to changes in pH, achieved by completely exchanging the sensing reservoir with buffers of different pH (pH = 8.0 initially, and pH = 9.0 and 10.0, at times = 0 and 57.25s, respectively) is shown in Figure A.8. The operating point for this device is $V_{DS} = 0.5$ V and $V_{GD} = 0$ V. The $I_{DS}$ of the n-type In$_2$O$_3$ nanowires decreases with increasing pH due to the decreasing degree of protonation of the surface hydroxyl groups. Fluid injection induces transients that settle to a steady state within 20s. Devices respond linearly to unit steps in pH in the pH 8-10 range.

![Device response to unit changes in pH](image)

**Figure A.8 Device response to unit changes in pH.** For time < 0, a pH 8.0 buffer was present in the reservoir. At time = 0 (red arrow) this buffer was exchanged with a buffer at pH 9.0 and at time = 57.25 s (green arrow) a second exchange, with a pH 10.0 buffer, was performed. All buffers were 0.01X PBS with 150 mMNaCl and were titrated using NaOH and HCl.

In order to calibrate the device’s pH response, we apply suggested method of calibration by measuring device solution transconductance ($g_m$) which describes device gating magnitude as a
function of solution gate electrode. Around 4ul of 0.1X PBS with 137mM of NaCl was added to
the mixing chamber and device electrical characteristics were recorded. Gating of the device was
achieved through the solution by submerging an Ag/AgCl reference electrode. Figure A.9
demonstrates our measurement setup (left) and typical transfer characteristics (device current
versus solution gate, right).

![Figure A.9](image)

**Figure A.9** a) Schematics of a measurement setup used for recording the solution gating
characteristics of a nanowire FET. (b) Current-solution gate characteristics of a representative
device.

The effect of solution gating on a representative device is given on Figure A.9. The device-to-
solution leakage current ($I_{LEAKAGE}$) remains two orders of magnitude below the device current
($I_{DS}$) for $V_{GD} \leq 0.8$ V. Thus, to calculate solution $g_m$ at the operating point i.e. $V_{GS}=0$V, a linear
best fit is made to the solution-phase $I_{DS}$ vs. $V_{GD}$ plot for $0 \leq V_{GD} \leq 0.3$ V. Device sensitivity
shows a clear correlation between increasing pH and solution $g_m$, demonstrated in Fig. 3.8 for
four different devices. A linear fit to these data yields a trendline that is within 6.7% error of the
value of each $I_{DS}/\Delta pH$ data point. Device sensitivity can be calculated by dividing $\Delta I/\Delta pH$ unit by
the solution $g_m$. The average sensitivity of the four devices shown in Figure A.10 is $18.2 \pm 1.2$
mV/pH unit, a value below the maximum sensitivity, which is the ideal Nernst potential of 58
mV/pH unit

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Figure A.10 Scatter plot of the $\Delta I_{DS}$/pH unit response vs. the solution transconductance ($g_m$) values for four In$_2$O$_3$ nanowire devices, as indicated in the legend.

The ne-ELISA

Operation of the ne-ELISA is demonstrated in A1.5. The reservoir was half filled (2 µL) with the sensing buffer (0.01X phosphate buffered saline, PBS, plus 150 mM sodium chloride) at an initial pH = 8.0. Devices were stabilized for 5-10 min under active measurement conditions ($V_{DS} = 0.5V$, $V_{GD} = 0V$). This equilibration time was required for the channel current ($I_{DS}$) to reach a steady state and is similar to that required for the elimination of initial background current in conventional ISFET glucose sensors. During sensing measurements, 2 µL of a 100 µM urea solution in the same pH 8.0 buffer was manually added with a micropipette and the solution was mixed by micropipette mixing for ~5-10 s. Introduction of this solution occurred at time = 0 in all figures. The response of a device to the presence of 12.5 pg/mL solution of IL-2 is shown in Figure A.11. The decrease in $I_{DS}$ and its continued negative derivative indicated that the addition of the urea solution resulted in a continuous drop in pH throughout the course of the measurement. In a conventional ELISA, the readout signal should be linear in time; here, it is observed to slow. This decrease in slope over time is most likely due to a product of both the
slowing of enzyme activity with increasing pH, and the pH-dependant deviation (decrease) from ideal Nernstian behavior of an oxide surface.

Figure A.11 Response $[I_{DS}(time)]$ of the sensor configured for IL-2 detection with 25 pg/mL IL-2 present during the protein-binding step (Figure A1.3-iii). At $time = 0$ the 100 µM urea solution was added to the pH 8.0 buffer. For this device, $\Delta I_{pH} = 68.0$ nA. The dashed red and green lines show the initial and final $I_{DS}$ levels, respectively.

The key detection parameter is the asymptotic current difference ($\Delta I_{pH}$), calculated by subtracting $I_{DS}(time \geq 40$ s) from $I_{DS}(time < 0$ s), thus the transient current spikes observed during urea addition and subsequent mixing do not interfere with the assay. For the device shown in Fig.A1.7, $\Delta I_{pH} = 68.0$ nA. To demonstrate that this decrease in $I_{DS}$ was due to urease activity and not to addition of the urea solution, a control device without bound urease was used. Upon introduction of the urea solution, a decrease of 1.8 nA in $I_{DS}$ was observed, thus setting this value as the assay’s lower sensitivity limit.

Detection sensitivity of the ne-ELISA was determined by treating devices with decreasing concentrations of IL-2. We measured device responses to seven serial dilutions of IL-2, starting with a concentration of 100 pg/mL. The responses of the seven devices (and one control) were converted into $\Delta$pH changes by fitting the solution transconductance values of the devices
(determined as described above after sensing measurements were completed) to the trendline determined from the control devices in Figure A.10. Due to the nature of the assay, each device could be used only once, thus each data point is derived from a single device. Reproducibility of device response is not a concern since each device is individually calibrated prior to sensor use. The ∆pH versus IL-2 concentration is plotted in Figure A.12 and demonstrates the sensitivity of the assay as low as <1.6 pg/mL and in the range of IL-2 concentrations relevant for T cell stimulation. We note that the ∆pH versus IL-2 concentration calibration curve is non-linear, primarily because pH is a logarithmic scale (−log[H⁺]) versus linear analyte concentration (and to a lesser degree, the decrease in activity and response with increasing pH).

![Figure A.12 Plot of the ∆pH measured by eight devices vs. the IL-2 concentration incubated with each sensor (left). The red circle derives from a control device to which no IL-2 was added during the protein-binding step (Figure A1.3-iii). The dotted box shows the zoomed part on the right.](image-url)
A2. Estimating protein charge dependence on pH

Using a UniProt library of protein sequences and the Scripps Institute Protein calculator one can estimate the amount of charge on the protein at different pH levels. It is important to notice that the value for electron charge is only approximate since the simulation does not take into account the steric effects on the pKₐ values of amino-acids but rather their native form. Figure A.13 shows the dependence of the total charge of the protein (CA15.3) on pH level of the buffer solution. At pH 9 which was the value used in the experiments this values is approximately -10.2e.

![Figure A.13 Total charge dependence of protein charge on pH level of the buffer solution. Approximate calculation is based on the Scripps Institute Protein Calculator.](image)

The peptide sequence of the CA15.3 (from the UniProt online library) is:

```
MTPGTQSPFLLLLLTVLTVTSGHASSTPGGEKETSATQRSSVPSSTEKNAVSMTSSVLSSHPGSGSSTTQGQDVTLA
PATEPASGAATWQDVTSPVTRPALGSTTPPAHDTVTSADNKAPAGSTAPPAGHGTSA
PDTARPAGSTAPPAGHGTSA
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